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16. ABSTRACT

The EPA has recognized the need for a rational approach to the incorporation of ecological impact information into power facility siting decisions in the northern great plains. Research funded by the Colstrip, Coal-Fired Power Plant project is a first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. Since then, key characteristics of the ecosystem have been monitored regularly to detect possible pollution impacts upon plant and animal community structure.

In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models for SO_2 stress on a grassland ecosystem. These experiments involve continuous stressing of one acre grassland plots with measured doses of SO_2 during the growing season (usually April through October).

Results of the 1979 field season's investigations are summarized in this publication. This is the last interim report of the six year project. Final reports will be published in 1981.

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Sixth Interim Report, Colstrip, Montana

August, 1980

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PREFACE

The Environmental Protection Agency has funded research for use in assessing potential impacts of coal-fired power plant siting in the northern Great Plains. Planners need to be able to predict environmental impacts of power plants when evaluating construction proposals. Power plant managers need environmental monitoring methods to warn of developing ecological damage in time for mitigation efforts to be effective. The project rationale and design was presented in detail in introductory sections of previous interim reports.

The project activities include monitoring of ecological effects from two 350 megawatt coal-fired power plants at Colstrip, Montana as well as field and laboratory process studies designed to elucidate the mechanisms responsible for ecological effects from power plant emissions.

Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. This documentation continued until Colstrip generating unit 1 began operation in September, 1975. Since then key characteristics of the ecosystem have been monitored regularly to detect possible pollution impacts.

In 1974, a Zonal Air Pollution System (ZAPS) was designed to stress 0.5 hectare areas of native grassland with measured concentrations of SO_2. In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models of SO_2 stresses on a grassland ecosystem. A second Zonal Air Pollution System (ZAPS II), was constructed in 1976. Both ZAPS I and ZAPS II were operated during the growing seasons of 1976, 1977, 1978, and 1979. The design of these experimental systems has been described in previous interim reports. Their behavior is further described in the present report.

Methodologies used in evaluating the effects of SO_2 on grassland plants and animals is presented in Sections 1 through 3 of the report. Sections 4 through 12 report results of research concerning the effects of SO_2 exposure on energy flow and nutrient cycling in grasslands, Sections 13 through 17 describe the effects of chronic SO_2 exposure on population and community structure, and Sections 18 through 23 describe research concerning potentially useful bioindicators and biomonitors of coal-fired power plant emissions.

ABSTRACT

The grasslands of the northern plains consist of a complex combination of interacting plant and animal species that capture, store, and utilize energy in a self-sustaining manner. Pollution stress on these rangelands may affect a variety of ecological processes.

The Coal-Fired Power Plant Project at Colstrip is designed to evaluate the environmental impact of air emissions from coal-fired power plants in the northern Great Plains ecosystems. The project consists of three parts:

(1) A case study of the ecological impact of two 350 megawatt coal-fired generating units at Colstrip, Montana.

(2) A series of field and laboratory process studies designed to elaborate the mechanism of SO_2 action on grasslands chronically fumigated at low levels.

(3) Development of a methodology for incorporating ecological effects information into the power plant siting process.

Documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. This continued until Colstrip generating Unit 1 began operation in September, 1975. Since then, key characteristics of the ecosystem have been monitored to detect air pollution and its ecological effects. This report summarizes the results of the 1979 field season.

Methodology

In 1974, a Zonal Air Pollution System (ZAPS) was designed to stress 0.5 hectare areas of native grassland with measured concentrations of SO_2. Field stressing experiments were initiated during the summer of 1975. A second ZAPS was constructed in 1976. Both ZAPS I and ZAPS II were operating during 1976, 1977, 1978, and 1979.

During the 1979 field season, the geometric mean SO_2 concentrations were 0.8-1.4, 2.4-2.6, 4.9, and 7.3 pphm on ZAPS I and 0.9-1.4, 2.4-2.6, 5.0-5.1, and 9.5-9.7 pphm on ZAPS II for the Control, Low, Medium, and High treatments, respectively. These concentrations were similar to those of past years of fumigation.

For laboratory experiments an exposure chamber is described for studies of long-term (2-4 months) SO_2 effects on organisms (*i.e.* grasshoppers and microorganisms) involved in plant litter decomposition. Also described is a relatively inexpensive physiological activity and diagnostic chamber designed to measure foliar exchange rates of SO_2, H_2O, and CO_2 in grass leaves.

Energy Flow and Nutrient Cycling

Several field and laboratory studies were conducted to evaluate the effects of SO_2 exposure on energy flow and nutrient cycling in the native grassland ecosystem. The experiments were specifically designed to provide information required to improve simulation models of SO_2 effects on system processes that are under development.

In an exposure chamber experiment, translocation of photoassimilated $^{14}CO_2$ by individual source leaves of *Agropyron smithii* were stimulated by exposure of the plants to 10 pphm SO_2. Leaves near the top of the plant supplied proportionately more assimilate to the developing leaf than to rhizomes. Lower leaves partitioned a greater proportion to a SO_2 stimulated rhizome sink. Sulfur dioxide exposure increased ^{14}C concentrations in the belowground compartment only on the first sampling date. The initiation of tillers is probably similar to that of developing leaves and requires carbon import until self-sustaining photosynthetic capacity is reached.

A laboratory chamber experiment exposing western wheatgrass litter to 8.5 pphm for 5 weeks showed a 9-17 percent reduction in decomposition rates under SO_2 exposure compared to controls. Lowered pH conditions in the litter may have been responsible for reduced microbial activity.

It was hypothesized that SO_2 exposure of the ZAPS sites was stressing the western wheatgrass population and that by subjecting the population to an additional stress (simulated grazing), a large negative effect on the population would be observed. Results of a field experiment failed to support this hypothesis. The only responses observed could have resulted from the simulated grazing alone and did not suggest synergism with SO_2 exposure level.

Bouteloua gracilis, an important warm season grass native to the Great Plains of North America, was grown hydroponically on Low, Medium, and High treatments on the ZAPS. After 31 days exposure, plants showed no treatment effect on live shoot weights, net weights, shoot:root ratios, or number of tillers. However, crown weight significantly decreased and live:dead shoot ratios increased on the lowest treatment plot and decreased on Medium and High treatments.

Leaching by precipitation influences estimates of sulfur accumulation rates. Such leaching from western wheatgrass tillers on the ZAPS High treatment was estimated to be 5-13 percent depending upon the time of exposure. Over-winter loss of plant sulfur was estimated to be 54 and 74 percent for Control and SO_2 exposed tillers, respectively. Accounting for rainfall leaching increased ability to predict live plant sulfur concentration by 6 percent.

Chlorophylls *a* and *b* in eight species of grassland plants exposed to SO_2 on ZAPS were increased, unchanged, or decreased depending upon SO_2 concentration and species. Chlorophyll *a* was most sensitive to exposure but the degree of sensitivity was species specific.

Sulfur distribution in western wheatgrass exposed to SO_2 on the ZAPS High treatment under various root sulfur availability regimes was studied to determine the interactive effects of atmospheric and substrate sulfur on sulfur metabolism. Sulfur dioxide exposure had no effect on shoot and root sulfur content. Root sulfur availability significantly affected plant sulfur content. Shoot/root ratios were greater early in the season on SO_2 exposed plants but the trend late in the season suggested reduced root growth rather than increased shoot growth. Nitrogen fertilization of western wheatgrass exposed to SO_2 on the ZAPS treatments appeared to ameliorate the effects of SO_2 exposure and increased the rate of senescence with increasing levels of SO_2 exposure.

Soil samples from the ZAPS High treatment had 6 times the sulfur content of those from the Control plot.

Population and Community Structure

Germination and establishment in the greenhouse were significantly reduced on soils from the High treatment for five grassland species. Nongraminoids were particularly reduced. Volunteer weeds were more abundant and diverse on soils from the Control and Low treatment plots than on the Medium and High treatment plots.

Neither nematodes nor rotifers were measurably affected by SO_2 exposure on the ZAPS plots, though soil microarthropod groups were significantly reduced. These reductions were not large enough to affect the total microarthropod population distribution. Most population reduction occurred in the first half of the growing season when soil water conditions were highest. Significant population reductions were also observed in some groups of aboveground arthropods, but these were not large enough to cause a significant change in overall aboveground arthropod population.

An exposure chamber study of the effects of 17 pphm SO_2 on the migratory grasshopper *Melanoplus sanguinipes* (Fab.) showed no significant difference between Control and SO_2 exposed individuals in egg viability, mean developmental time for each nymphal instar, adult dry weight biomass, and egg production per female per day. This suggests that previously reported population reductions of this species on certain ZAPS treatments were not due to direct toxic effects. The reported population reductions may have resulted from migration off the treatment sites.

Bioindicators and Biomonitors

Lichens are used throughout the world as bioindicators of air quality. Since 1975, various characteristics of native lichens have been monitored at various distances from Colstrip. In 1979, respiration rates, chlorophyll content, and rate of photosynthesis in two lichen species (*Usnea hirta* and *Parmelia chlorochroa*) had no significant linear relationship with distance from Colstrip. However, sulfur content of *Usnea hirta* significantly decreased with increased distance from Colstrip. If Colstrip emissions have had any impact on lichens in the vicinity, it has been slight.

Anatomical and physiological responses of *Usnea hirta* and *Parmelia chlorochroa* transplants on the ZAPS plots were measured to determine SO_2 dose/response relationships for these two species. Significant reductions in respiration rate in *Usnea hirta* occurred on the Low plot after 100 days exposure. Reductions in pigment content and increases in plasmolyzed algal cells occurred within 90 days. On the Medium treatment, respiration rates decreased and algal cell plasmolysis increased within 60 days. *Parmelia chlorochroa* exposed near the soil surface showed no detectable response.

Managed honey bees appear promising for use as biological monitors of coal-fired power plant emissions. Since 1974, honey bees from commercial apiaries in the Colstrip area have been sampled and analyzed for fluoride, a toxicant released by coal combustion.

Honey bees in the vicinity of Colstrip, Montana showed significant increases from fluoride levels in 1977 and 1978. Patterns of fluoride in pollen and the geographical distribution of fluoride buildup with respect to Colstrip and prevailing winds suggest airborne rather than waterborne fluoride exposure.

Baseline histological information on western meadowlark and the deer mouse was gathered for later comparison with data gathered after power plant operation. These histological studies describe seasonal cycles in body composition, organ system function, and energetics.

Western meadowlark lungs collected in the vicinity of Colstrip, Montana were examined for particulates during 1975 (before plant operation), 1976, 1977, and 1978. Three types of particles were observed including crystals of variable size, very small ($\leq$ 0.47 um) round black flecks and large black particles of round or irregular shape. Particulate burdens increased steadily between 1975 and 1977 and were similar for male and female birds. Juveniles had a smaller burden than adults.

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METHODOLOGY

SECTION 1

TEMPORAL VARIATION IN SO_2 CONCENTRATIONS ON ZAPS DURING 1979

T. J. McNary

ABSTRACT

The Zonal Air Pollution System (ZAPS) was designed to deliver atmospheric SO_2 pollution on a small portion of the prairie ecosystem in Montana. Geometric mean SO_2 concentrations were 0.82-1.41, 2.44-2.59, 4.88-4.92, and 7.32-7.35 pphm for the Control and Low-, Medium-, and High-SO_2 plots, respectively, on ZAPS I and 0.87-1.39, 2.42-2.64, 5.01-5.15, and 9.52-9.73 pphm on ZAPS II. Except for the high-SO_2 plot, SO_2 concentrations were similar to those of past years of fumigation. Diel patterns showed much greater SO_2 concentrations at night than during the day, and indicated that diel activity patterns of organisms must be considered when evaluating dose-response relationships.

INTRODUCTION

The ZAPS consisted of two 11-ha exclosures in the mixed-grass prairie of southeastern Montana, in the Ashland Division of Custer National Forest. The system was designed for experimental evaluation of the effects of long-term, low-level exposure to sulfur dioxide. Parameters evaluated included plant and animal community structures, insect population and behavior, pollination systems, lichens, physiological functions, and biochemical functions.

Each exclosure contained four 0.5-ha plots, each receiving a different fumigation level of SO_2. The four plots were designated control (A), Low (B), Medium (C), and High (D) to describe the relative level of SO_2 received. The SO_2 was distributed over each plot through a network of aluminum pipes suspended about 75 cm above the soil surface. Gas-release orifices (0.8 mm dia.) were positioned at 3-m intervals, with more than 250 orifices on each plot. No locus on a plot was more than 5.5 m from an orifice. A detailed description of the design of the ZAPS is presented by Lee *et al.* (1976), Lee and Lewis (1978), Lee *et al.* (1979), and Preston *et al.* (1980).

Although controls did not receive SO_2 through the network of pipes, the controls were exposed to SO_2 that drifted from upwind plots. Consequently, controls received low-level, periodic SO_2 fumigation that resembled exposure from a point source rather than from an area source. Sulfur dioxide was applied to treatment plots throughout the growing season (April-October); fumigation began on ZAPS I in May 1975 and on ZAPS II in April 1976. Sulfur dioxide exposure was at a constant rate but concentrations within plots varied with meterological conditions, primarily wind speed.

In this section temporal variation in SO_2 concentration is characterized.

MATERIALS AND METHODS

Sulfur dioxide concentration was measured at a central locus on each plot with a Meloy Laboratory Sulfur Analyzer (Model SA 160-2) on a time-sharing basis throughout the season. Ambient air samples were measured at 35 cm above the ground, which is close to canopy height for this type of grassland.

In general, the procedures described in Lee *et al.* (1979) modified by Preston *et al.* (1980) were followed in 1979. Span checks of the analyzer were performed semi-weekly, and calibrations adjusted when needed.

Calibration curves that related analyzer response to logarithms of SO_2 concentration became nonlinear at concentrations below 2 pphm. Therefore, linear extrapolation led to an overestimation of SO_2 levels in the 0-2 pphm range. True values were probably between 0.1 pphm and the value estimated from the calibration curve. During data analysis, each analyzer response was entered into two calibration equations. The High run was adjusted to yield a maximum value that could result from the above-mentioned sources of uncertainty. The Low run yields the corresponding minimum value for the SO_2 concentration. Summary statistics were computed for both the High and Low runs.

RESULTS AND DISCUSSION

Interseasonal Trends

A seasonal summary of SO_2 concentration for ZAPS I and ZAPS II for 1979 is presented in Tables 1.1 and 1.2. The first number for each entry is for the Low run value and the second number is for the High run value. If only one number appears, there was no difference between the Low and High run values.

Sulfur dioxide concentrations for the Control, Low, and Medium plots in 1979 were similar to those in past years (Table 1.3). For Low-SO_2 plots, concentrations declined slightly from the peaks in 1977. Trends of increasing SO_2 on High plots over the years continued for ZAPS II and reached a geometric mean of 9.52-9.73 pphm in 1979. This trend was dramatically reversed on the ZAPS I High-SO_2 plot, where the SO_2 concentration decreased to a geometric mean of 7.32-7.35 pphm. For more information on SO_2 concentrations in past years, see Preston *et al.* (1980) for 1978 data and Lee *et al.* (1979) for 1977 and 1976 data.

TABLE 1.1. ZAPS I SEASONAL SUMMARY, 1979*

Treatment	Geometric mean	Standard geometric deviation	Arithmetic mean
Control	0.82-1.41	3.49,1.63	1.47-1.69
	1-hour ave. exceeded 25 pphm	0 times:	0 percent
	3-hour ave. exceeded 50 pphm	0 times:	0 percent
	24-hour ave. exceeded 10 pphm	0 times:	0 percent
	24-hour ave. exceeded 14 pphm	0 times:	0 percent
	1-hour peak: 19.12	3-hour peak: 13.24	24-hour peak: 5.25
Low	2.44-2.59	2.43,3.49	3.70-3.73
	1-hour ave. exceeded 25 pphm	19.0 times:	.48 percent
	3-hour ave. exceeded 50 pphm	0 times:	0 percent
	24-hour ave. exceeded 10 pphm	2.0 times:	1.10 percent
	24-hour ave. exceeded 14 pphm	0 times:	0 percent
	1-hour peak: 47.41	3-hour peak: 27.79	24-hour peak: 13.47
Medium	4.88-4.92	2.55,2.49	8.52
	1-hour ave. exceeded 25 pphm	203.0 times:	5.09 percent
	3-hour ave. exceeded 50 pphm	1.0 times:	.07 percent
	24-hour ave. exceeded 10 pphm	40.0 times:	21.98 percent
	24-hour ave. exceeded 14 pphm	13.0 times:	7.14 percent
	1-hour peak: 87.49	3-hour peak: [illegible].65	24-hour peak: 28.41
High	7.32-7.35	2.41,2.37	11.62
	1-hour ave. exceeded 25 pphm	471.0 times:	11.82 percent
	3-hour ave. exceeded 50 pphm	18.0 times:	1.33 percent
	24-hour ave. exceeded 10 pphm	91.0 times:	50.00 percent
	24-hour ave. exceeded 14 pphm	49.0 times:	26.92 percent
	1-hour peak: 138.12	3-hour peak: 88.80	24-hour peak: 37.92

* pphm SO_2 monitored near treatment center at canopy height.

TABLE 1.2. ZAPS II SEASONAL SUMMARY, 1979*

Treatment	Geometric mean	Standard geometric deviation	Arithmetic mean
Control	0.87-1.39	2.89,1.27	1.24-1.45
	1-hour ave. exceeded 25 pphm	0 times:	0 percent
	3-hour ave. exceeded 50 pphm	0 times:	0 percent
	24-hour ave. exceeded 10 pphm	0 times:	0 percent
	24-hour ave. exceeded 14 pphm	0 times:	0 percent
	1-hour peak: 19.43	3-hour peak: 10.34	24-hour peak: 2.34
Low	2.42-2.64	2.56,2.07	3.78-3.82
	1-hour ave. exceeded 25 pphm	5.0 times:	0.18 percent
	3-hour ave. exceeded 50 pphm	0 times:	0 percent
	24-hour ave. exceeded 10 pphm	2.0 times:	1.18 percent
	24-hour ave. exceeded 14 pphm	1.0 times:	0.59 percent
	1-hour peak: 39.78	3-hour peak: 25.71	24-hour peak: 18.13
Medium	5.01-5.15	2.71,2.50	8.57-8.58
	1-hour ave. exceeded 25 pphm	168.0 times:	6.19 percent
	3-hour ave. exceeded 50 pphm	0 times:	0 percent
	24-hour ave. exceeded 10 pphm	40.0 times:	23.53 percent
	24-hour ave. exceeded 14 pphm	14.0 times:	8.24 percent
	1-hour peak: 60.65	3-hour peak: 47.36	24-hour peak: 33.40
High	9.52-9.73	2.97,2.77	18.13-18.14
	1-hour ave. exceeded 25 pphm	487.0 times:	17.93 percent
	3-hour ave. exceeded 50 pphm	61.0 times:	5.19 percent
	24-hour ave. exceeded 10 pphm	119.0 times:	70.00 percent
	24-hour ave. exceeded 14 pphm	83.0 times:	48.82 percent
	1-hour peak: 152.72	3-hour peak: 113.58	24-hour peak: 56.00

* pphm SO_2 monitored near treatment center at canopy height.

TABLE 1.3. ANNUAL GEOMETRIC MEAN SO_2 CONCENTRATION (PPHM) FOR ZAPS I AND ZAPS II, 1975-79

		Treatment			
Year	Site	Control	Low	Medium	High
1975	ZAPS I	1.0	1.7	3.8	6.7
1976	ZAPS I	0.2-0.9	1.8-2.2	3.7-3.9	6.5-6.7
	ZAPS II	0.2-1.4	2.6-2.9	4.1-4.4	6.4-6.9
1977	ZAPS I	0.2-1.6	3.2-3.4	5.3-5.6	7.0-7.5
	ZAPS II	0.4-1.6	2.9-3.0	4.7-4.9	7.5-8.2
1978	ZAPS I	1.0-1.1	2.8	5.1	8.6-8.9
	ZAPS II	0.9-1.2	2.5	4.6	9.2-9.3
1979	ZAPS I	0.8-1.4	2.4-2.6	4.9	7.3-7.4
	ZAPS II	0.9-1.4	2.4-2.6	5.0-5.2	9.5-9.7

The frequency of high concentrations was less in 1979 than in 1978, except for the High plot on ZAPS II, which was similar to that of 1978 (Figure 1.1) (Preston *et al.*, 1980; Lee *et al.*, 1979). The data were presented in this format for comparison with previous reports.

Intraseasonal Trends

Intraseasonal trends on ZAPS I and ZAPS II were different. On ZAPS I there was a slight increase in SO_2 concentration on all plots throughout the growing season (Figure 1.2). On ZAPS II Control, Low, and Medium plots, SO_2 concentration remained relatively constant. Concentration on the High plot decreased from April to June, then increased for the remainder of the season (Figure 1.3).

Diel Patterns

Diel patterns of SO_2 concentration on each ZAPS site were similar in 1979 to previous years. Concentrations of SO_2 were lowest during the daylight hours (Figure 1.4 and 1.5), probably because of higher wind speeds and greater mixing of the air than at night.

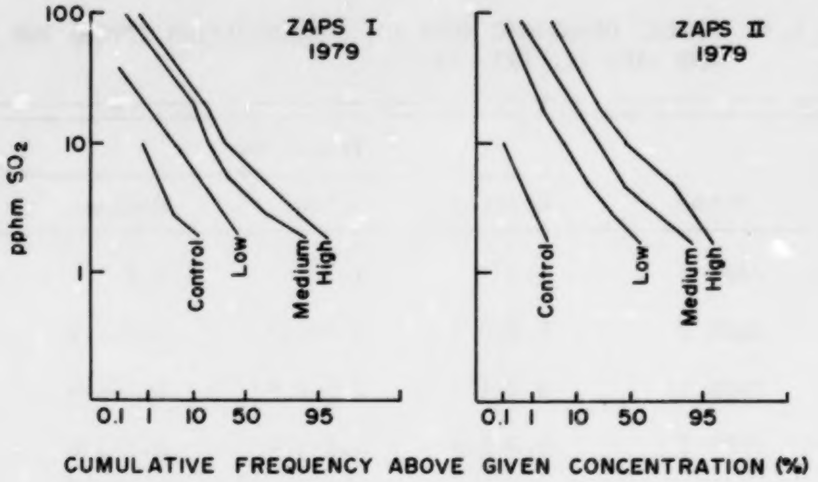

Figure 1.1. Frequency distribution of SO_2 concentration on ZAPS, 1979.

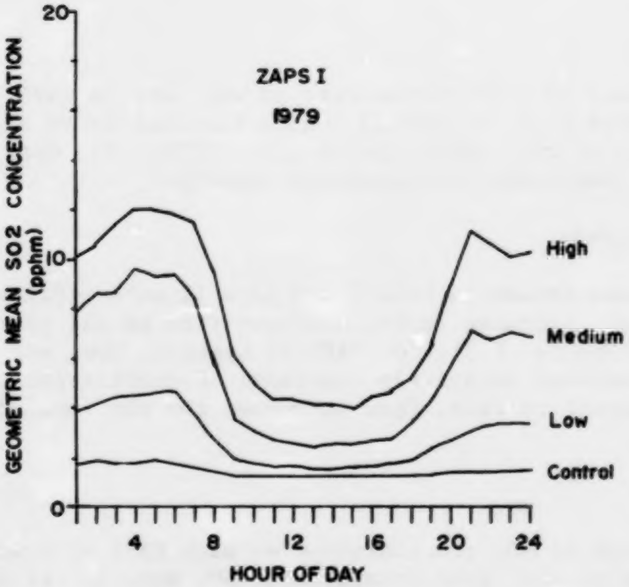

Figure 1.2. Monthly variation in geometric mean SO_2 concentration on ZAPS I, 1979 (High run).

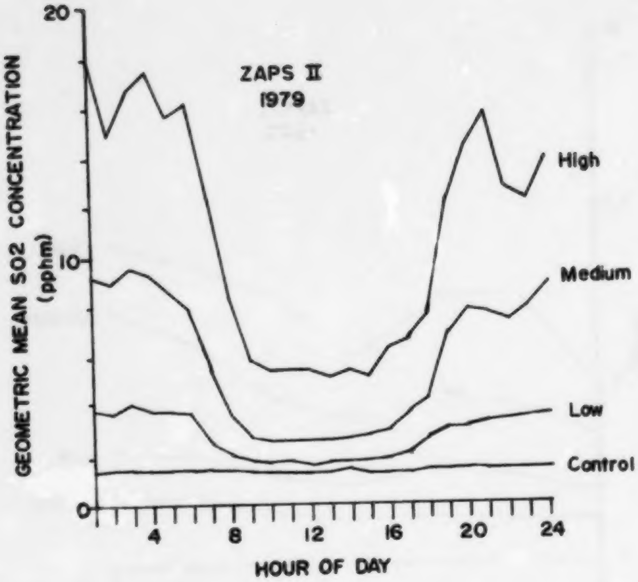

Figure 1.3. Monthly variation in geometric SO_2 concentration on ZAPS II, 1979 (High run).

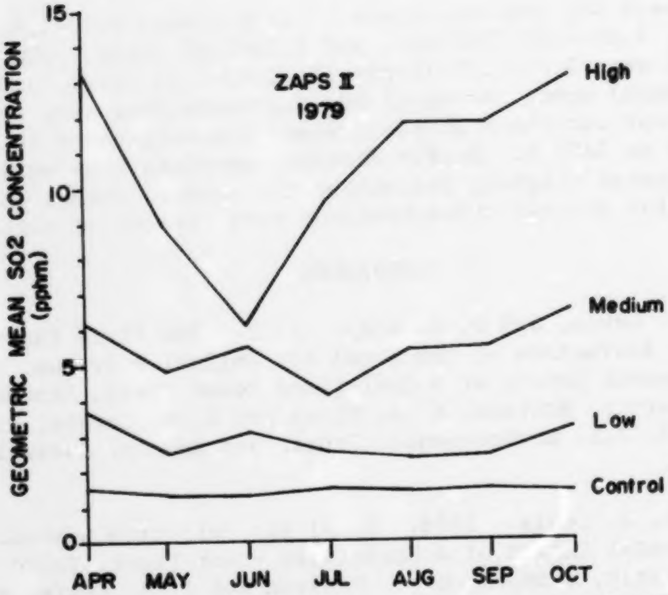

Figure 1.4. Diel cycles of SO_2 concentration on ZAPS I, 1979 (seasonal geometric mean, High run).

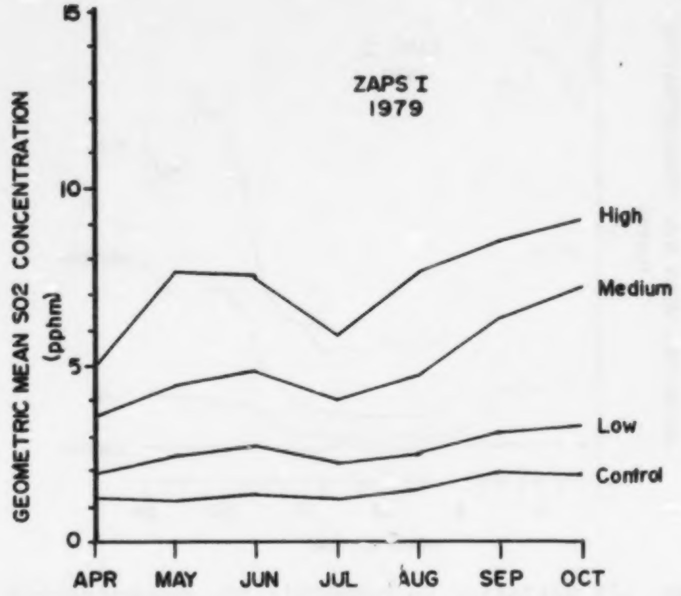

Figure 1.5. Diel cycles of SO_2 concentration on ZAPS II, 1979 (seasonal geometric mean, High run).

CONCLUSIONS

Geometric mean SO_2 concentrations on ZAPS I were 0.82-1.41 (Control), 2.44-2.59 (Low), 4.88-4.92 (Medium), and 7.32-7.35 (High) pphm. Concentrations on ZAPS II were 0.87-1.39 (Control), 2.42-2.64 (Low), 5.0-5.15 (Medium), and 9.52-9.73 (High) pphm. Seasonal SO_2 concentrations were similar to previous years, except for the High plot, where concentrations increased on ZAPS II and decreased on ZAPS I. Sulfur dioxide concentrations were relatively constant or increased slightly throughout the season, except for the High plot on ZAPS II. Sulfur dioxide concentrations were greater at night than during the day.

REFERENCES

Lee, J. J., R. A. Lewis, and D. E. Body. 1976. The Field Experimental Component: Evaluation of the Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Second Interim Report, Colstrip, Montana. N. R. Glass and A. S. Lefohn, eds. EPA 600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 188-202.

Lee, J. J., and R. A. Lewis. 1978. Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Third Interim Report, Colstrip, Montana. E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 322-344.

Lee, J. J., E. M. Preston, and D. B. Weber. 1979. Temporal Variation in SO_2 Concentration on ZAPS. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA 600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 284-305.

Preston, E. M., T. L. Gullett, and D. B. Weber. 1980. Temporal Variation in SO_2 Concentrations on ZAPS During the 1978 Field Season. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn, eds. EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 96-107.

SECTION 2

DESIGN AND CONSTRUCTION OF A SIMPLE, CONTINUOUS FLOW SULFUR DIOXIDE EXPOSURE CHAMBER

J.W. Leetham, W. Ferguson, J.L. Dodd, and W.K. Lauenroth

ABSTRACT

The design and construction of a low-cost, low-maintenance SO_2 exposure chamber is described. The chamber is designed to be used for organisms other than plants and for long exposure periods. Efficiency of the chamber is discussed.

INTRODUCTION

Most studies of the effects of air pollutants on organisms and/or biological processes require exposure to controlled levels of the pollutants for specific time periods. Since most controlled-exposure studies deal with plants, the requirements of a system to administer the pollutant exposure are quite stringent in regards to environmental conditions which affect growth of the test plants. These requirements often lead to the construction of expensive exposure chambers (Heck *et al.*, 1978), especially if critical short-term exposures and physiological response measurements are involved.

Studies of air pollutants and other air-born toxicants have encouraged the design of a wide array of controlled-exposure chambers covering a spectrum from very simple (Berry and Ripperton, 1963) to extremely sophisticated (Heck *et al.*, 1978). Berry (1970) categorized the various chamber designs into four general groups: 1) small modified greenhouses; 2) simple plastic enclosures for use in greenhouse or laboratory; 3) modified growth chambers; and 4) plastic chambers designed to fit in and utilize the environmental control of a growth chamber. The first two groups may utilize only natural light while the last two require artificial light. Examples of the various groups can be found in Adams (1961), Berry and Ripperton (1963), Berry (1970), Cantwell (1968), Costonis (1968), Heck *et al.* (1968), Heck *et al.* (1978), Hill (1967), Hitchcock *et al.* (1963), Katz *et al.* (1939), Lockyer *et al.* (1976), Menser and Heggestad (1964), Thomas *et al.* (1943), McLaughlin *et al.*, Heagle and Philbeck (1979), and Zimmerman and Hitchcock (1956). To Berry's list (Berry 1970) may be added a fifth group which includes outdoor, open topped chambers for studying ambient air pollutants under near natural conditions (Heagle *et al.*, 1973).

Heagle and Philbeck (1979) discussed various requirements of exposure chambers used in studies of plants and air pollutants. They concluded that a chamber should have a continuous flow, single pass air stream containing the desired pollutant. The chamber walls should be inert to the pollutant and as non-adsorptive of the pollutant as possible. For plants, light transmitting qualities of the chamber walls must be carefully considered if external lighting is used. Depending on the type of study, temperature and relative humidity conditions may or may not be critically controlled and the level at which these two conditions are to be maintained will govern the type and/or quality of materials and equipment used within the chamber. The most critical characteristic of any chamber is the stability and homogeneity of the pollutant concentration in space and time. The mode of introducing the pollutant must be stable over time and the movement of the air stream must not create eddies or dead spots within the chamber. Turbulent rather than laminar air flow is generally desirable.

For our experimental purposes, a reasonably large capacity, low cost, low maintenance chamber was needed to study the long-term (2-4 months) effects of sulfur dioxide on developmental rates of grasshoppers and decomposition rates of plant litter. Internal temperature, humidity, and light controls were not required since the chamber would be used in externally controlled environments. However, the chamber needed to satisfy most of Heagle and Philbeck's (1979) conditions.

CHAMBER DESIGN

The chamber (Figure 2.1) was constructed completely of 0.64 cm ($^1/_4$") thick plexiglass to allow use of ambient light conditions. Since racks of grasshopper rearing containers were to be used, the side-opening double doors were made large for easy access to the chamber. Weatherstripping was used to seal around doors. Air movement through the chamber was accomplished with a push-pull system using an inlet and an exhaust fan. The inlet fan air movement capacity was rated at 25 percent of the exhaust fan ($1.7\ m^3 \cdot min^{-1} = 60$ cfm). This system created a negative internal pressure to prevent leakage. Fans were simple squirrel-cage types (Dayton Blowers, Dayton Electric Mfg. Co., Chicago). The shroud on the inlet fan was removed to produce a symmetrical dispersion of SO_2-air mixture. The SO_2 was introduced directly into the incoming air stream as it entered the inlet fan. To maximize the uniformity of airflow through the chamber to the exhaust end, the inlet orifice of the exhaust fan was directed to the downwind end of the chamber. Sulfur dioxide concentrations were measured through access holes drilled in the chamber top at three points downwind. At each point, holes were positioned to allow SO_2 measurement with a stainless steel probe at numerous points in a cross-sectional plane. Point measurements of SO_2 concentrations were made with a flame photometric sulfur gas analyzer (Meloy Laboratories Inc., Model SA-160). During the actual studies, SO_2 concentrations were monitored by a chemical absorption technique (using pararosanaline dye) which gave average concentrations over time (CFR, 1975).

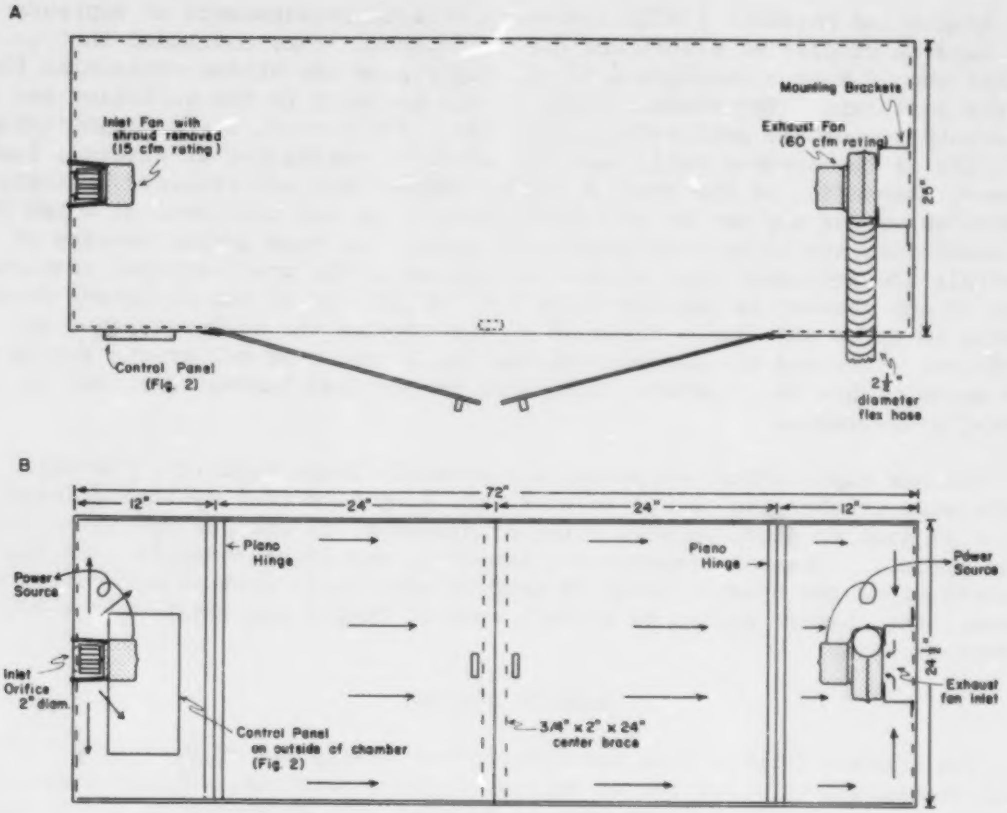
A
Inlet Fan with shroud removed (15 cfm rating)
Mounting Brackets
Exhaust Fan (60 cfm rating)
25"
Control Panel (Fig. 2)
2½" diameter flex hose
B
72"
12"
24"
24"
12"
Piano Hinge
Piano Hinge
Power Source
Power Source
Inlet Orifice 2" diam.
Control Panel on outside of chamber (Fig. 2)
3/4" x 2" x 24" center brace
24½"
Exhaust fan inlet

Figure 2.1. Diagram of SO_2 exposure chamber. A) top view, B) front view. Arrows indicate air flow.

Since our objective for constructing the chamber was long-term exposure capabilities, a low-cost, low-maintenance source of SO_2 gas was needed. A small lecture bottle of liquid SO_2 proved quite adequate. The gas was injected into the inlet fan air stream with the delivery system diagramed in Figure 2.2. Because the metering valve was very sensitive to changes in line pressure as a result of temperature changes on the SO_2 lecture bottle, the bottle was kept in a refrigerated ice bath to keep temperature fluctuations to a minimum. The ball valve was used to shut off gas flow without affecting the metering valve settings once a desired SO_2 delivery rate was established. A spring-loaded solenoid was installed on the ball valve handle to prevent SO_2 buildup in the chamber in the event of an electrical power failure.

OPERATION EFFICIENCY

Air turnover in the chamber was calculated to be once every 1 to 2 minutes which produced an approximate linear velocity of 0.46 to 0.91 m $\cdot$ min^{-1}

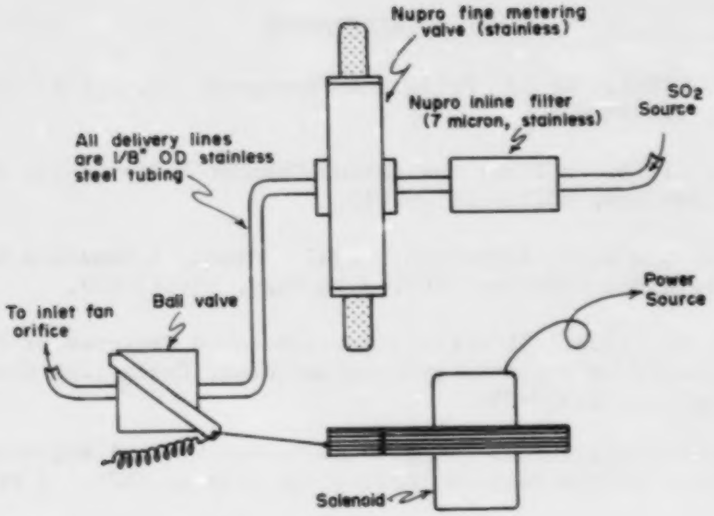
Nupro fine metering valve (stainless)
Nupro inline filter (7 micron, stainless)
SO2 Source
All delivery lines are 1/8" OD stainless steel tubing
Power Source
To inlet fan orifice
Ball valve
Solenoid

Figure 2.2. Schematic of control panel.

(1.5-3.0 ft · min^{-1}). Air flow rates were altered by either altering the inlet or exhaust fan orifices or changing either fan size. The exposure system as constructed was capable of producing relatively stable SO_2 concentrations between 5 and 100 pphm although the lower and upper limits were not determined.

Because the weatherstripping used to seal the doors did not make an absolute seal, there was slight variation of SO_2 concentrations both laterally and longitudinally. At an exhaust concentration of 20 pphm there was a 3 to 5 pphm longitudinal gradient with the greatest decrease being from the inlet fan to the hinge of the first door. This upwind 30% of the chamber was considered as a dilution-mixing zone and was not used for experimental purposes. From the first door to the exhaust fan the SO_2 gradient was less than 2 pphm. The variation (max-min) of SO_2 concentration at a given cross-sectional plane was less than ±18 percent of the mean concentration for that plane. This was true for three different concentration settings - 20, 17, and 8.5 pphm mean concentration at the exhaust fan inlet. The variation of SO_2 concentration at the exhaust fan inlet over time was quite small - less than 1 pphm over long term intervals (up to 2 months) and less than 0.5 pphm over short intervals (24 hours).

CONCLUSIONS

The controlled exposure chamber herein described has proven to be adequate for studies involving long-term SO_2 effects on organisms such as grasshoppers and micro-organisms involved in plant litter decomposition. It satisfied most of the basic conditions discussed by Heagle and Philbeck (1979). Its utility could be increased by use within an environmentally controlled greenhouse. It is comparatively simple and inexpensive to construct and maintain.

REFERENCES

Adams, D. F. 1961. An Air Pollution Phytotron. J. Air Pollut. Control Assoc., 11:470-476.

Berry, C. R. 1970. A Plant Fumigation Chamber Suitable for Forestry Studies. Phytopathology, 60(11):1613-1615.

Berry, C. R., and L. A. Ripperton. 1963. Ozone, A Possible Cause of White Pine Emergence Tipburn. Phytopathology, 53:552-557.

Cantwell, A. M. 1968. Effect of Temperature on Response of Plants to Ozone as Conducted in a Specially Designed Plant Fumigation Chamber. Plant Dis. Reptr., 52;957-960.

Code of Federal Regulations (Supplement to the Federal Register). Title 40 - Protection of Environment, Part 50.11 July 1, 1975. 7 pp.

Costonis, A. C. 1968. The Relationships of Ozone, *Lophodermium pinastri*. and *Pullularia pullulans* to Needle Blight of Eastern White Pine. Ph.D. Thesis, Cornell University, Ithaca, New York. 167 p.

Heagle, A. S., D. E. Body, and W. W. Heck. 1973. An Open-top Chamber to Assess the Impact of Air Pollution on Plants. J. Environ. Quality, 2(3):365-368.

Heagle, A. S., and R. B. Philbeck. 1979. Exposure Techniques, In: W. W. Heck, S. V. Krupa, and S. N. Linzon (eds.) Metholodology for the Assessment of Air Pollution Effects on Vegetation. Air Pollution Control Association, Pittsburg, Pa. pp. 6-1 to 6-19.

Heck, W. W., J. A. Dunning, and H. Johnson. 1968. Design of a Simple Plant Exposure Chamber. USDHEW Nat. Center Air Pollution Control Pub. APTD-68-6 24 p.

Heck, W. W., R. B. Philbeck, and J. A. Dunning. 1978. A Continuous Stirred Tank Reactor (CSTR) System for Exposing Plants to Gaseous Air Contaminants: Principles, Specifications, Construction and Operation. Agricultural Research Service, U. S. Dept. Agriculture, ARS-S-181 32 p.

Hill, A. C. 1967. A Special Purpose Plant Environmental Chamber for Air Pollution Studies. J. Air Pollut. Control Assoc., 17(11):743-748.

Hitchcock, A. E., P. W. Zimmerman, and R. R. Coe. 1963. The Effect of Fluorides on Milo Maize (Sorghum sp.) Contrib. Boyce Thompson Inst., 22:175-206.

Katz, M., A. W. McCallum, G. A. Ledingham, and A. E. Harris. 1939. Description of Plots and Apparatus used in Experimental Investigations, In: Effect of Sulphur Dioxide on Vegetation. Nat. Res. Council Can. No. 815, Ottawa. pp. 207-217.

Lockyer, D. R., D. W. Cowling, and L. H. P. Jones. 1976. A System for Exposing Plants to Atmospheres Containing Low Concentrations of Sulphur Dioxide. J. Exper. Bot., 27(98):397-409.

McLaughlin, S. B., V. J. Schorn, and H. C. Jones. 1979. A Programmable Exposure System for Kinetic Dose-response Studies with Air Pollutants. J. Air Pollut. Control Assoc., 26(2):132-135.

Menser, H. A., and H. E. Heggestad. 1964. A Facility for Ozone Fumigation of Plant Materials. Crop. Sci., 4:103-105.

Thomas, M. D., R. H. Hendricks, J. O. Ivie, and G. R. Hill. 1943. An Installation of Large Sand-culture Beds Surmounted by Individual Air-conditioned Greenhouses. Plant Physiol., 18:334-344.

Zimmerman, P. W., and A. E. Hitchcock. 1956. Susceptibility of Plants to Hydrofluoric Acid and Sulfur Dioxide Gases. Contrib. Boyce Thompson Inst., 18:263-279.

SECTION 3

A SYSTEM FOR MEASURING FOLIAR EXCHANGE RATES UNDER ENVIRONMENTALLY CONTROLLED CONDITIONS

S. R. Bennett, J. L. Dodd, W. S. Ferguson, C. J. Bicak,
C. L. Thor, and W. K. Lauenroth

ABSTRACT

A relatively inexpensive physiological activity and diagnostic chamber designed to measure foliar exchange rates of SO_2, H_2O, and CO_2 in grass leaves is described. The system consists of gas synthesis, cuvette, linkage, gas flow control and monitoring elements. Equations for calculating gas flux rates are included.

INTRODUCTION

Investigations of the effects of gaseous air pollutants on plants take many forms (Heck *et al.*, 1979). Some research objectives are satisfied by measurements of frequency of plant injury or percentage of foliar necrosis while other research objectives require measurement of physiological changes in response to particular pollutant exposure regimes (Tingey *et al.*, 1979). The latter approach is particularly suited to investigations of pollutant mediated plant responses on a short term basis, or when exposure to low concentrations of known toxic agents is not reflected in visible injury.

The deleterious effects of SO_2 on vegetation have been widely documented since its recognition as a chronic gaseous pollutant in industrialized areas (Ziegler, 1975). Despite considerable research, anomalous physiological responses to SO_2 exposures not resulting in foliar injury and variable species sensitivity to similar SO_2 concentrations remain unexplained (Thor, 1980). The purpose of this paper is to describe a relatively inexpensive experimental system designed to monitor foliar exchange rates of CO_2 and water vapor under a range of controlled SO_2, relative humidity, and temperature conditions.

The system described below was used to expose grass leaves to SO_2 concentrations between 10 and 100 pphm, relative humidity between 4.0 and 75.0 percent and temperatures between 5 and 28°C. The apparatus consisted of a compressed air supply which provided the carrier airstream through the system, a gas synthesis subsystem, a multileaf cuvette, instrumentation to measure CO_2 SO_2, dew point, temperature and quantum irradiance, a data acquisition subsystem, linkage, and control points for gas flow regulation. This system is the type of experimental apparatus termed "a physiological activity and diagnostic chamber" by Bennett (1979). It is similar in principle to the system described by Winner and Mooney (1980).

MATERIALS AND METHODS

Experimental Cuvette

Following a design similar to one described by Williams and Kemp (1978) and Detling *et al.* (1979), a circular Plexiglass leaf cuvette 16.4 cm in diameter was constructed. The device consisted of two fitted sections, forming a 3.6- cm high chamber surrounded by upper and lower water jackets for temperature regulation (Figure 3.1). The water jackets were connected to a circulating bath equipped with flow rate and temperature control. A magnetic stir bar in the lower chamber of the cuvette insured a homogenous air mixture and reduced boundary layer resistance. Boundary layer resistances were 0.2 ± 0.1 s $\cdot$ cm^{-1} in a system of similar design and operational protocol (Kemp, 1977).

The cuvette was sealed by compressing a narrow bead of nontoxic sealing compound (Mortite) between the two cuvette sections. Grass leaves were enclosed between the fitted sections and rested on fine nylon line strung above the stir bar. In addition to providing an airtight seal, the mortite functioned as a protective cushion which completely surrounded the leaves. Air temperature within the cuvette was continuously monitored with a shaded YSI thermistor (Model 421). Leaf temperatures were not measured but were assumed to be equivalent to air temperatures within the cuvette. Detling (unpublished) tested a cuvette of similar design and operational protocol and found leaf temperatures of well-watered plants to be within 1°C of cuvette air temperatures. Irradiance was supplied by a 1,000 watt Westinghouse Ceramalux high pressure sodium lamp suspended on a pulley directly above the cuvette. Quantum irradiance maintained at 1250-1350 μE m^{-2} s^{-1} was measured with a LI-170 Quantum sensor photometer and was controlled by varying the distance between lamp and cuvette. A water screen placed directly beneath the sodium lamp aided in reducing heat transfer to the cuvette.

Gas Synthesis

Initially air was obtained from a central compressor in the laboratory and was carried through the gas exchange system in heavy wall 1/4" Tygon tubing. This was supplemented where necessary with short lengths of 1/4" stainless steel, $^1/_4$" copper and $^1/_4$" glass tubing. Carbon dioxide was eliminated by passing the airstream through a concentrated potassium hydroxide trap, and molecular sieves (Matheson Model 460 Gas Purifier) placed in series removed oil and water contamination. Last, a column of desiccant and a column of ascarite (NaOH, A. H. Thomas Company) placed in series removed remaining traces of water vapor and CO_2, respectively (Figure 3.2). The air obtained from the laboratory compressor contained variable amounts of CO_2 and moisture and was later replaced with medical grade reconstituted air. The cylinders of reconstituted air provided an air supply which was uniformly dry and CO_2 free. Utilization of reconstituted air also eliminated the need for extensive

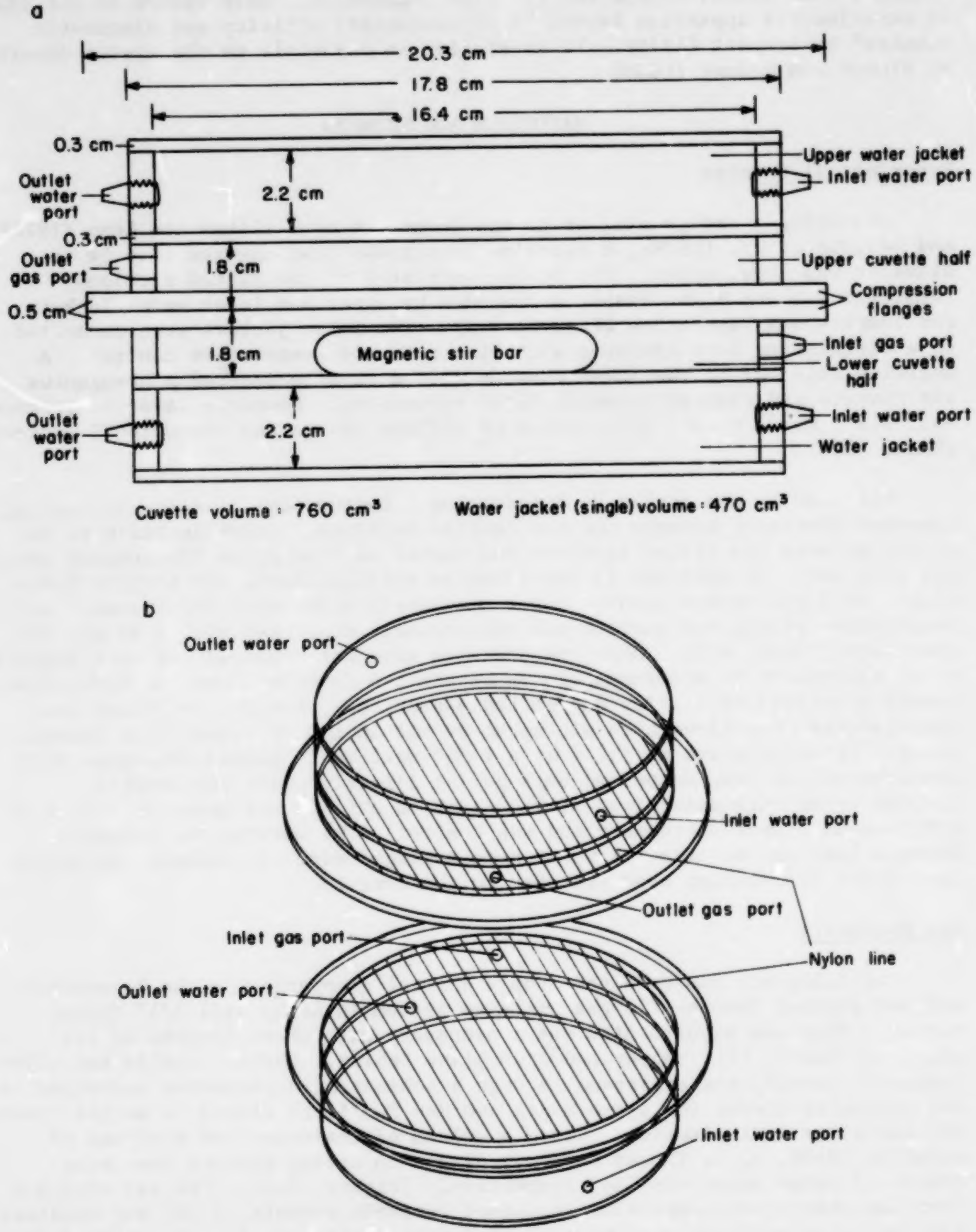
a
20.3 cm
17.8 cm
16.4 cm
0.3 cm
Outlet water port
2.2 cm
Upper water jacket
Inlet water port
0.3 cm
Outlet gas port
1.8 cm
Upper cuvette half
0.5 cm
Compression flanges
1.8 cm
Magnetic stir bar
Inlet gas port
Lower cuvette half
Outlet water port
2.2 cm
Inlet water port
Water jacket
Cuvette volume : 760 cm³
Water jacket (single) volume : 470 cm³
b
Outlet water port
Inlet water port
Outlet gas port
Inlet gas port
Nylon line
Outlet water port
Inlet water port

Figure 3.1. Experimental plexiglass cuvette design.

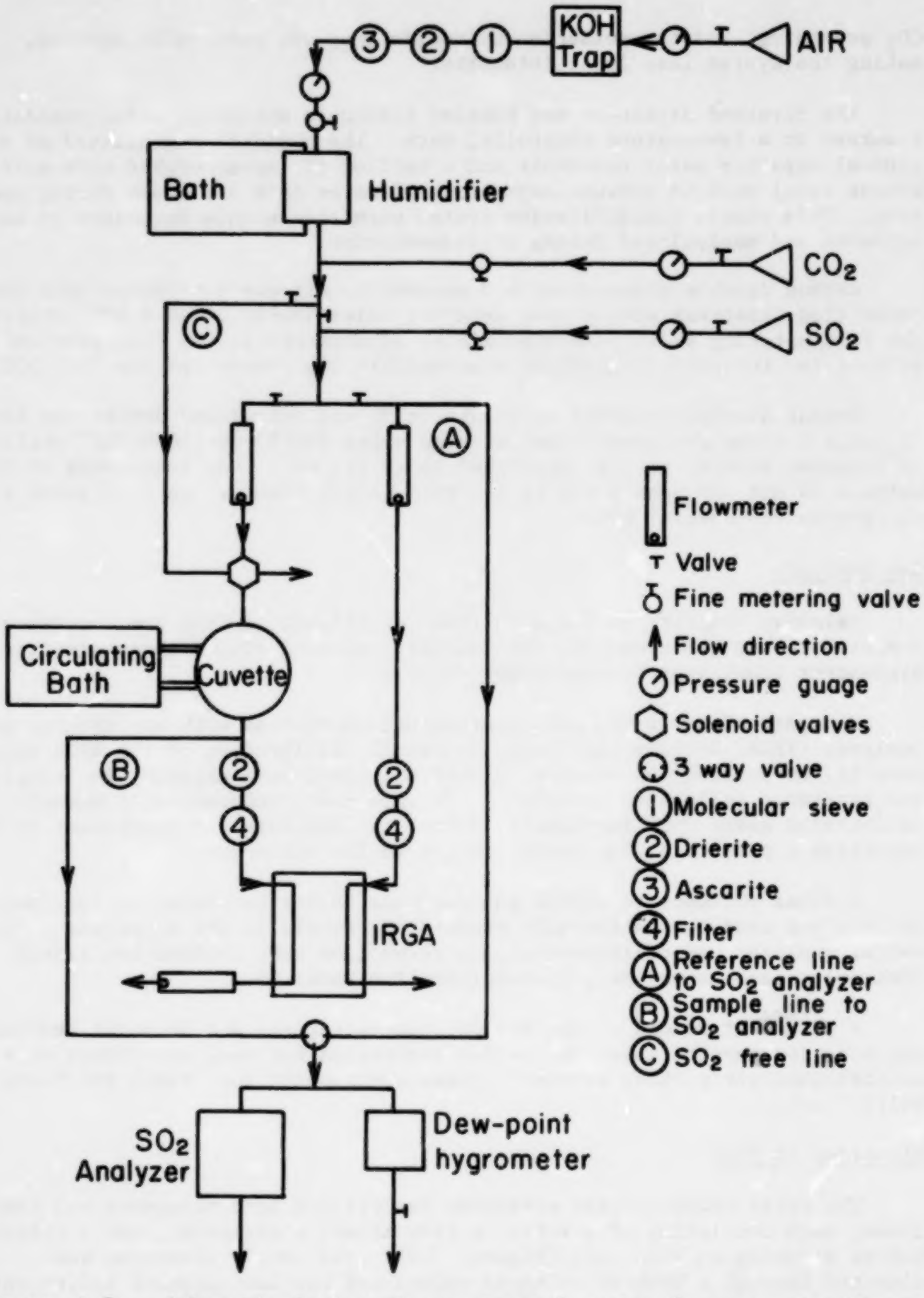
KOH Trap
AIR
③ ② ①
Bath
Humidifier
CO_2
SO_2
Ⓒ
Ⓐ
Circulating Bath
Cuvette
Ⓑ
② ④
② ④
IRGA
SO_2 Analyzer
Dew-point hygrometer
Flowmeter
Valve
Fine metering valve
Flow direction
Pressure guage
Solenoid valves
3 way valve
① Molecular sieve
② Drierite
③ Ascarite
④ Filter
Ⓐ Reference line to SO_2 analyzer
Ⓑ Sample line to SO_2 analyzer
Ⓒ SO_2 free line

Figure 3.2. Schematic design of experimental system for measuring fluxes of CO_2, SO_2 and water vapor in grass leaves.

CO_2 scrubbing, and the potassium hydroxide trap was eventually removed, making the system less labor intensive.

The scrubbed airstream was bubbled through a stainless steel humidifier immersed in a temperature controlled bath. The humidifier consisted of a 1500-ml capacity water reservoir and a baffled air space packed with sufficient coarse steel mesh to prevent aspiration of water into air lines during operation. This simple humidification system permitted stable dewpoints to be achieved and manipulated during experimentation.

Carbon dioxide prepared at 4.0 percent in air was introduced into the humidified airstream with a fine metering valve (NUPRO B-45G 0.031" orifice). The fine metering valve permitted minute adjustments in CO_2 flow rate and allowed for the establishment of reproducible CO_2 concentrations (ca. 330 ppm).

Sulfur dioxide prepared at 50 ppm in N_2 was introduced downstream from CO_2 with a stainless steel fine metering valve (NUPRO-SS-45G 0.031" orifice) in a manner similar to that described above for CO_2. Concentrations of SO_2 between 10 and 100 pphm could be achieved within minutes and maintained in the system for several hours.

Gas Analysis

Relative humidity in the airstream was calculated from the cuvette air temperature and the dewpoint, continuously measured with a thermoelectric hygrometer (Cambridge Systems Model 880-C1).

Concentrations of CO_2 were continuously monitored with an infrared gas analyzer (IRGA, Beckman Instrument Company). Calibration of the IRGA required manually by-passing the cuvette so that the airstreams entering the sample and reference cells were identical. In this mode, independently measured CO_2 calibration gases were introduced, either to calibrate the instrument or to establish a particular CO_2 concentration in the airstream.

A flame photometric sulfur gas analyzer (Meloy Laboratories Inc. Model SA-160) was used to continuously measure SO_2 levels in the airstream. The sulfur analyzer was calibrated with a permeation tube calibration system (Metronics Associates Inc., Dyna-calibration Model 330).

A permanent record of CO_2 and SO_2 concentrations and dewpoint temperatures was made for cuvette inlet and outlet airstreams for each experiment on a multichannel strip chart recorder (Texas Instruments Inc. Model FMW SE-60 Multiriter).

Direction of Flow

The fully reconstituted airstream was divided into reference and sample lines, each consisting of a valve, a flow meter, a desiccant, and a filter before entering an IRGA cell (Figure 3.2). The sample airstream was directed through a bank of solenoid valves and the leaf cuvette before entering the IRGA sample cell. After exiting the IRGA, the sample airstream passed through an additional flow meter which was used to detect leakage from the cuvette. The reference airstream was directed into an exhaust port after exiting the IRGA reference cell.

Two additional air lines (Figure 3.2, A and B), diverted portions of the reference and sample airstreams to a 3-way valve (Whitey B-43XF4 0.187" orifice). This valve was used to manually direct the airstreams alternately through both the sulfur analyzer and dewpoint hygrometer. Changes in the cuvette environment were detected by alternate measurement and comparison of the two airstreams.

Just prior to the entry point for SO_2, another air line (Figure 3.2, C) diverted a portion of the airstream to the bank of solenoid valves located just above the cuvette. The air in this line remained SO_2-free, but in all other respects was identical with the fully reconstituted airstream. The solenoid valves permitted instantaneous routing of either the sample or SO_2-free airstream through the cuvette, automatically diverting the unused airstream to an exhaust port. Routing SO_2-free air through the cuvette allowed SO_2 concentrations to be achieved and stablized in the sample and reference lines without disrupting the environmental equilibrium of the cuvette. This arrangement also made it possible to instantaneously expose the cuvette to stable, predetermined levels of SO_2 without first isolating the cuvette from the system. Flow rate in the SO_2-free line was established prior to full reconstitution of the airstream and continued unchanged for the duration of each experiment.

Regulation of Flow

Flow rates in the gas exchange system were regulated at three principle points. The flow rate of scrubbed air entering the system was controlled by a fine metering valve (Nupro B-4MG 0.055" orifice) located just above the humidifier (Figure 3.2). Flow rates in the reference and sample lines could be adjusted during operation with individual valves located at the beginning of each line. Generally, a flow rate ($1.0\ l\ m^{-1}$ $1.5\ l\ m^{-1}$) sufficient to maintain a 1500-2500 pphm CO_2 differential between the sample and reference lines was established at the beginning of an experiment and continued unchanged for its duration. The fine metering valve controlling the supply of scrubbed air could not be changed during an experiment since any change in flow rate at this point in the system would disrupt the established ratio of gases.

Calculations

Rates of photosynthesis and transpiration uptake were represented by the following gas flux equations (Winner and Mooney, 1980).

$$J_x = \Delta_x FA^{-1} t$$

where

J_x = flux rate for any gas (mass area^{-1} time^{-1})
Δ_x = change in gas concentration due to experimental leaf tissue ($\mu l\ l^{-1}$)
F = air flow rate ($l\ m^{-1}$)

A = area of experimental leaf tissue (dm^2)

t = 60 min

Appropriate constants for local temperature and atmospheric pressure were multiplied into the above calculation.

When Δ_x measurements were made for CO_2, and water vapor,

$$J_x = J_p, J_t,$$

thus

$J_x = J_p$ = net photosynthesis ($\mu g\ CO_2\ dm^{-2}\ hr^{-1}$)

$= J_t$ = transpiration ($g\ H_2O\ dm^{-2}\ hr^{-1}$)

Stomatal conductance values for water vapor (C_w) and SO_2 (C_s) were calculated as

$$C_w = 1/R_w$$

$$R_w = \frac{(H_i - H_a) \cdot \rho \cdot 3.6 \times 10^5}{J_t}$$

R_w = resistance to water vapor ($s \cdot cm^{-1}$)

H_i = specific humidity of air in substomatal chamber

H_a = specific humidity of air outside leaf

ρ = air density

J_t = transpiration

3.6×10^5 = conversion factor for units

and

$$C_s = C_w \cdot \frac{D_s}{D_w}$$

where

C_w = conductance for water vapor

D_w = diffusivity coefficient for water vapor (0.2475)

D_s = diffusivity coefficient for SO_2 (0.1313)

Flux of SO_2 through the stomates ($\mu g \cdot cm^{-2} \cdot s$) was then calculated as

$$F_s = \frac{S_a - S_i}{R_s}$$

where

$R_s = \frac{1}{C_s}$

S_a = SO_2 concentration in air around leaf (exit concentration)

S_i = SO_2 concentration in substomatal chamber (assumed to be 0)

Operational Protocol and System Performance

The system was applied to attached leaves of several species of grass plants according to the following general procedure:

1. Preliminary calibration and adjustments were made on all gas synthesizing, environmental control, and monitoring equipment.
2. Two or three healthy leaves of each plant were sealed into the cuvette.
3. Final adjustments were made on all control points to attain precise environmental conditions prescribed for a given trial.
4. The system was then allowed to equilibrate until inlet and outlet dewpoints, CO_2 uptake rates, and cuvette air temperatures were stable for at least 30 min. The stable period was considered the control.
5. Simultaneous with Step 4, SO_2 was introduced into an airstream bypassing but otherwise identical to that flowing into the cuvette. Sulfur dioxide concentration in the airstream was set to a specified level.
6. At the end of the control period the airstream containing SO_2 was routed into the cuvette and the SO_2-free airstream was directed to an exhaust manifold via a solenoid switching mechanism.
7. Exposure of the leaves to SO_2 was continued until CO_2 and H_2O flux rates attained an apparent stable rate for at least 30 minutes. In most trials the rates stabilized within about 15 to 20 minutes and the trial was terminated after 1 hr of exposure.

Results of two representative SO_2 exposure trials are shown in Table 3.1. In trial A, CO_2 flux and transpiration rates decreased to a stable rate within 10 min. Trial B leaves exhibited lower control photosynthetic rates and higher transpiration rates and did not stabilize until about 30 min into the exposure period. Stomatal flux rates of SO_2, being based on water vapor conductance rates and cuvette outlet concentrations of SO_2, closely paralleled rate dynamics of transpiration because the outlet SO_2 concentrations were reasonably stable through these runs.

TABLE 3.1. GAS EXCHANGE DYNAMICS AND ENVIRONMENTAL CONDITIONS FOR TWO EXAMPLE TRIALS (A AND B) UTILIZING ATTACHED LEAVES OF HYDROPONICALLY GROWN WESTERN WHEATGRASS PLANTS

	Control*		Elapsed time (min) from initiation of SO_2 exposure									
			10		20		30		60		120	
Trial	A	B	A	B	A	B	A	B	A	B	A	B
SO_2 concentration (pphm X 10^{-1})												
cuvette inlet	0	0	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.9	10.7
cuvette outlet	0	0	8.9	7.6	9.0	8.1	9.1	8.2	9.3	8.4	9.5	8.8
Air temperature (C°)	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9
Irradiance ($\mu E \cdot dm^{-2} \cdot s^{-1}$)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Absolute humidity (g $H_2O \cdot m^{-3}$ air)												
cuvette inlet	8.5	7.2	8.4	6.9	8.4	7.0	8.4	7.0	8.4	7.1	8.4	7.2
cuvette outlet	11.0	11.6	11.0	11.5	11.0	11.2	11.0	11.1	11.0	11.2	11.0	11.2
CO_2 concentration (ppm X 10^{-1})												
cuvette inlet	35.2	34.9	35.2	34.9	35.2	34.9	35.2	34.9	35.2	34.9	35.2	34.9
cuvette outlet	32.9	32.5	33.3	32.7	33.3	32.8	33.3	32.8	33.3	32.8	33.3	32.8
Flow rate ($l \cdot min^{-1}$ X 10^2)	95.0	95.0	96.0	95.0	96.0	95.0	96.0	95.0	96.0	95.0	96.0	95.0
Leaf area (cm^2 X 10)	90.7	101.8	90.7	101.8	90.7	101.8	90.7	101.8	90.7	101.8	90.7	101.8
Atmospheric pressure (mb X 10^{-1})	85.4	85.3	85.4	85.3	85.4	85.3	85.4	85.3	85.4	85.3	85.4	85.3
CO_2 flux (mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$)	22.0	20.5	18.4	18.8	18.4	18.4	18.4	17.9	18.4	17.9	18.4	17.5
Transpiration (g $H_2O \cdot dm^{-2} \cdot hr^{-1}$ X 10)	18.5	24.8	16.3	25.4	16.3	23.5	16.3	23.5	16.3	23.0	15.9	22.2
Stomatal flux of SO_2 (ng $SO_2 \cdot cm^{-2} \cdot s^{-1}$ X 10^2)	0	0	41.0	56.8	41.5	54.8	42.0	55.7	43.0	55.8	42.8	55.9

* Control data is from a 30 to 60 minute interval immediately preceding initiation of SO_2 exposure when all monitored parameters were stable.

Certain problems were encountered in utilizing this system in nearly 200 trials. The major problem was our inability to monitor dewpoint of the inlet and outlet airstreams precisely. The dewpoint hygrometer utilized is capable of determinations to the nearest 0.5°C. Consequently, our estimates of transpiration and stomatal flux of SO_2 were frequently variable and only of general value. The strong points of the system are that it permits fairly precise measurement of CO_2 exchange rates in grass leaves under a range of environmental conditions and is relatively inexpensive.

CONCLUSIONS

The gas exchange system described proved to be a relatively inexpensive but effective means to measure effects of low-level, short-duration SO_2 exposure on sulfur uptake, net photosynthesis, and transpiration in western wheatgrass leaves. Although SO_2 and H_2O exchange rates could only be measured rather crudely the performance characteristics of the system were generally very good. A rapid response time permitted several short-duration experiments at varying conditions to be conducted within a single 8-10 hr period. Although the system was incapable of measuring fluxes of CO_2, SO_2 and water vapor at the level of precision achieved by Winner and Mooney (1980) with their more sophisticated system, we conclude this system has utility in studies where objectives require less precision and where fiscal resources are more limited.

REFERENCES

Detling, J. K., M. I. Dyer, and D. T. Winn. 1979. Net Photosynthesis, Root Respiration, and Regrowth of *Bouteloua gracilis* Following Simulated Grazing. Oecologia, 41:127-134.

Bennett, J. H. 1979. Foliar Exchange of Gases. In: Methodology for Assessment of Air Pollution Effects, W. W. Heck, S. V. Krupa, and S. N. Linzon, eds. Air Pollution Control Association. Ch. 7.

Heck, W. W., S. V. Krupa, and S. N. Linzon, eds. 1979. Methodology for the Assessment of Air Pollution Effects on Vegetation. Air Pollution Control Association.

Kemp, P. R. 1977. Niche Divergence Between *Agropyron smithii*, C_3, and *Bouteloua gracilis*, C_4: A Study of the Role of Differing Photosynthetic Pathways in the Shortgrass Prairie Ecosystem. Ph.D. Dissertation. Washington State University, Pullman, Washington.

Thor, G. L. 1980. Photosynthesis in Western Wheatgrass Exposed to Chronic SO_2 Air Pollution. M.S. Thesis. Colorado State Univ., Ft. Collins. 71 pp.

Tingey, D. T., R. G. Wilhour, and O. C. Taylor. 1979. The Measurement of Plant Responses. In: Methodology for Assessment of Air Pollution Effects, W. W. Heck, S. V. Krupa, and S. M. Linzon, eds. Air Pollution Control Association. Ch. 7.

Williams, G. J., III, and P. R. Kemp. 1978. Simultaneous Measurement of Leaf and Root Gas Exchange of Shortgrass Priarie Species. Bot. Gaz., 139:150-157.

Winner, W. E., and H. A. Mooney. 1980. Ecology of SO_2 Resistance: I. Effects of Fumigations on Gas Exchange of Deciduous and Evergreen Shrubs. Oecologia.

Ziegler, I. 1975. The Effects of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79-105.

SECTION 4

THE EFFECT OF SO_2 ON ^{14}C TRANSLOCATION IN WESTERN WHEATGRASS

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ABSTRACT

Translocation of photoassimilated $^{14}CO_2$ by individual source leaves of *Agropyron smithii* Rybd. was stimulated by exposure of the plants on ZAPS I High plot. Labeled leaves exposed to SO_2 exported an average of 12 percent more carbon than control leaves. This was in response to an increased sink demand of expanding leaves. The relative concentration and relative partitioning of ^{14}C in developing leaves was 177 and 153 percent greater, respectively, in plants on the SO_2 treatment than in Control plants. Belowground ^{14}C concentrations were greater in SO_2 exposed plants only on the early growing season sampling date. Increased rhizome ^{14}C relative concentration suggests greater sink demand in reproductive as well as vegetatively growing components. a comparison is made between productivity, photosynthetic and ^{14}C partitioning responses in monitoring the subtle effects of low-level SO_2 exposure.

INTRODUCTION

The partitioning of photoassimilated carbon among various plant organs is an important aspect in the functioning of a plant as an integrated system. Translocation processes, and the maintenance of carbon balance, are subject to regulation involving both positive and negative feedback mechanisms (Geiger, 1979). The export of carbon from a source leaf is a function of the availability of sucrose and other mobile molecules, and the demand for these assimilates in other locations.

Plant nutrient levels can affect translocation independent of carbon assimilation rates. Increased levels of free-space potassium did not increase net carbon fixation but increased the proportion of fixed carbon which was exported (Geiger, 1979). Similar affects for levels of inorganic phosphorus and nitrogen were suggested by the observation that deficiences of phosphorus and nitrogen caused a decrease in the efflux, into solutions, of assimilated carbon from the mesophyll of beet leaves (Kamanina and Anisimov, 1977).

Sulfur dioxide exposure has been shown to affect photosynthesis, amino acid production, and carbohydrate metabolism in plants (Ziegler, 1975). Large quantities of sulfur accumulate in plants exposed to SO_2 depending on the concentration and duration of exposure and the environmental conditions during exposure (Lauenroth *et al.*, 1979). Short-term exposure to SO_2 has been shown to inhibit phloem translocation more than photosynthesis (Teh and Swanson, 1977). Exposure of plants to SO_2 then, may affect translocation directly as well as indirectly by its impact on assimilate supply/sink demand. In this study we examined the effect of a controlled level of SO_2 exposure through the growing season on translocation of ^{14}C in *Agropyron smithii* Rybd. in a native Montana grassland ecosystem. *Agropyron smithii* was dominant within the study area and is an important forage species throughout the northern Great Plains.

MATERIALS AND METHODS

Twelve western wheatgrass (*Agropyron smithii*) plants, with an equal number of fully expanded leaves, were chosen on the Control and High SO_2 treatment plots for labeling with $^{14}CO_2$. Three leaf age classes were labeled. Leaf numbers were assigned from lowest to highest on the plant; *i.e.* the oldest leaf was considered leaf number one. Leaf numbers three, four, or five were labeled on June 18 and 26, and leaf numbers five, six, or seven on July 15 and August 2 sampling dates.

Individual leaves were placed in a cuvette, sealed with putty, and exposed to $^{14}CO_2$ ($\sim$330 ppm) of specific activity 3.7×10^7 Bq $mmol^{-1}$ (1 mCi $mmol^{-1}$ for 2 minutes in full sunlight. After 3 days the plants were collected and separated into the three leaf age classes, the developing leaf, other aboveground leaves and stem, roots, and rhizomes. Roots were sampled to a depth of 10 cm. In a previous experiment on this study area, 62 percent of the belowground ^{14}C was located in roots from the 0-10 cm zone (Coughenour *et al.*, 1979). All samples were oven dried at 60°C to a constant weight.

The oven-dried samples were enclosed in Whatman low ash filter paper and compressed in a pill press to reduce their size. The plant material was wrapped in filter paper because grinding was impractical due to sample size and static electricity problems. Samples were then combusted in a Packard Instrument Co. model 306 Tri-carb sample oxidizer which automatically absorbed the $^{14}CO_2$ in Carbo-sorb II and placed it in a liquid scintillation vial with Permafluor V scintillation cocktail. Sample activities were counted on a Nuclear-Chicago Mark II liquid scintillation system.

The data are expressed as relative concentrations or relative quantities in each plant component. Relative concentration (CPM/mg of plant tissue) describes the sink strengths while relative amounts (CPM/total sample) of ^{14}C in each component directly addresses the movement of carbon. Because it was impossible to collect the entire belowground biomass for each labeled plant, discussions relating to aboveground/belowground are based only upon the concentration data (CPM/mg dry wt). Aboveground data are presented on both a sink strength and distribution basis.

Two aspects of our experimental design need clarification for proper interpretation of results. First, we refer to leaf age class rather than leaf age because both leaf age and the position of a leaf relative to carbon sinks can influence translocation (Wardlaw, 1968; Mor and Halevy, 1979). However, in an erect culm grass like *Agropyron smithii*, leaf position is a function of leaf age. Therefore, in this study leaf age and leaf position are not separable yet neither are they a source of possible interaction between plant replicates (*i.e.*, there is no possible age x position interaction). Second, leaf age classes remained constant for all dates. Date effects can only be attributed to overall plant phenology and the presence of additional and older leaves on the plant.

Data were subjected to an analysis of variance using a modified split plot design with subsampling. The main plot was SO_2 treatment and the split plot a 4 × 3 factorial of date by leaf. Replicates were arranged in randomized block fashion. Tukey's Q values were used to compute least significant ranges (LSR) and identify significant differences between means (Sokal and Rohlf, 1969).

RESULTS

Three days after exposure to $^{14}CO_2$, labeled leaves contained a higher concentration (CPM/mg) and quantity (CPM) of ^{14}C than other aboveground plant components regardless of date or SO_2 treatment (Table 4.1). Sulfur dioxide exposure significantly reduced the concentration ($P = 0.06$) and total quantity ($P = 0.01$) of ^{14}C remaining in labeled leaves indicating the presence of high demand in other plant components. Labeled leaves exposed to SO_2 exported an average of 12 percent more carbon than the leaves of control plants. Import of ^{14}C by mature unlabeled leaves accounted for 0.5 percent of the total aboveground ^{14}C in Control plants and 1 percent in those exposed to SO_2. Other aboveground plant parts, excluding the developing leaf, accounted for 17 and 22 percent of the aboveground ^{14}C for the Control and SO_2 treated plants, respectively.

The concentration ($P = 0.001$) and total quantity ($P = 0.05$) of ^{14}C remaining in labeled leaves was significantly influenced by the age class of the labeled leaf. Labeling the youngest, middle, and older fully expanded leaf resulted in ^{14}C concentrations which represented 79, 83 and 92 percent (LSR = 10) of the total aboveground concentration, respectively. Total quantities of ^{14}C retained in the labeled leaves showed the same pattern. The three leaf age classes from young to old retained 67, 77, and 84 percent (LSR = 11) of the total above ground activity. The large quantity exported from younger leaves may be a function of their age or proximity to the developing leaf.

In many cases the developing leaf was a strong carbon sink (Table 4.1). The concentrations and quantities of ^{14}C in developing leaves were 1.5 and 1.7 times greater, respectively, in SO_2 treated plants than control plants. Young leaves rely upon translocated carbon until they develop sufficient photosynthetic capacity to satisfy their needs. Swanson *et al.* (1976), Thrower (1962) and Fellows and Geiger (1974) reported that peak demand for carbon by developing leaves occurred when the leaf was approximately 25

TABLE 4.1. CARBON-14 RELATIVE CONCENTRATION AND PARTITIONING IN *AGROPYRON SMITHII* EXPOSED ON THE ZAPS I HIGH PLOT. VALUES ARE BASED ON MEANS FOR PLANTS HARVESTED 3 DAYS AFTER $^{14}CO_2$ ASSIMILATION

			^{14}C Mean Activity (%)					
			Labeled Old Leaf		Labeled Middle Leaf		Labeled Young Leaf	
Date	Treatment	Plant Part*	CPM/mg	CPM	CPM/mg	CPM	CPM/mg	CPM
June 18	Control	Leaf 3	97.0	95.4	0.2	0.2	0.1	<0.1
		Leaf 4	0.6	0.8	77.5	82.8	0.4	0.1
		Leaf 5	0.4	0.6	0.9	0.9	82.0	76.5
		Devl.Lf.	0.1	3.0	3.4	9.3	8.7	17.0
		Other	1.1	1.0	18.0	3.6	9.1	6.3
	SO_2	Leaf 3	89.0	74.9	0.4	0.2	0.2	0.1
		Leaf 4	1.1	1.4	84.5	78.5	0.3	0.2
		Leaf 5	0.8	1.0	1.2	1.1	64.1	43.1
		Devl.Lf.	6.8	20.8	10.1	18.1	13.4	46.8
		Other	2.3	2.0	3.9	2.8	22.1	9.8
June 26	Control	Leaf 3	97.6	95.0	0.7	0.5	0.3	0.3
		Leaf 4	0.2	0.2	91.6	78.5	0.9	0.9
		Leaf 5	0.5	0.5	1.2	0.8	82.8	61.9
		Devl.Lf.	1.2	3.9	5.4	19.4	8.5	32.4
		Other	0.5	0.5	1.2	0.8	7.5	4.6
	SO_2	Leaf 3	91.3	87.7	1.9	1.6	0.5	0.5
		Leaf 4	1.6	1.5	74.9	67.7	0.3	0.3
		Leaf 5	0.8	0.8	2.4	3.0	64.3	64.2
		Devl.Lf.	2.9	10.5	6.2	20.1	8.2	25.2
		Other	3.4	1.9	14.6	7.7	26.9	9.0
July 15	Control	Leaf 5	95.2	87.2	0.8	0.7	0.2	0.2
		Leaf 6	1.1	1.0	89.8	76.4	0.4	0.3
		Leaf 7	0.3	0.2	2.1	1.6	90.1	77.1
		Devl.Lf.	3.2	11.5	6.2	20.9	5.7	20.9
		Other	0.2	0.1	1.1	0.4	3.6	1.6
	SO_2	Leaf 5	91.1	86.5	0.3	0.4	0.6	0.6
		Leaf 6	2.8	2.9	74.5	82.6	1.5	1.4
		Leaf 7	0.9	0.7	1.8	1.7	88.8	84.3
		Devl.Lf.	2.8	9.4	2.6	9.7	3.2	10.8
		Other	2.4	0.6	20.8	5.7	5.9	2.8
August 2	Control	Leaf 5	94.0	78.5	0.5	0.5	0.1	0.1
		Leaf 6	0.6	0.5	91.9	81.7	0.7	0.7
		Leaf 7	0.3	0.2	1.1	0.7	90.7	70.2
		Devl.Lf.	4.7	20.8	6.2	17.1	7.6	28.9
		Other	0.5	0.1	0.3	<0.1	0.8	0.1
	SO_2	Leaf 5	84.3	65.3	0.7	0.5	0.4	0.5
		Leaf 6	2.8	2.2	80.7	64.7	0.9	0.8
		Leaf 7	0.9	0.5	2.5	1.6	73.0	57.8
		Devl.Lf.	11.0	31.8	14.9	32.9	10.0	36.3
		Other	0.9	0.2	1.2	0.3	15.7	4.6

* Leaf counts are from bottom to top of plant. The highest number represents the youngest fully expanded leaf. Devl. Lf. refers to the new developing leaf. Other refers to the stem and leaves other than the 3 top leaves and the developing leaf.

percent of its final length. We investigated the possibility that the greater ^{14}C activity in developing leaves on the SO_2 treatment was a function of leaf size rather than a treatment effect.

Mean weights and the distribution of weights for the developing leaves between the Control and the SO_2 treatment were very similar (Figure 4.1). These data were subjected to the same analysis of variance model used to examine ^{14}C partitioning and no significant differences ($P = 0.80$) were observed in leaf weight between the Control and SO_2 treatment. Differences in developmental stage were not significant for any three or two way interactions or for main effects with the exception of date. Weights of expanding leaves by date were 20.8, 18.4, 15.4, and 10.1 (LSR 8.7) for June 18, 26, July 15, and August 2, respectively. This suggested a decline in weight of expanding leaves as the season progressed rather than an unequal distribution of weights sampled. Further, in all our analyses of variance, date was a significant factor in date × treatment interactions rather than as a main effect. The analysis of variance for expanding leaves indicated that although weights declined as the season progressed, they remained uniform within a date-between-treatments or labeled-leaf-age classes. The greater translocation in plants on the SO_2 plots was therefore in response to SO_2 exposure and not because of nonuniform sizes of developing leaves between treatments or labeled leaf age classes.

The balance between above- and belowground sink strengths was modified by the interactions between treatment and date ($P = 0.01$) and between treatment

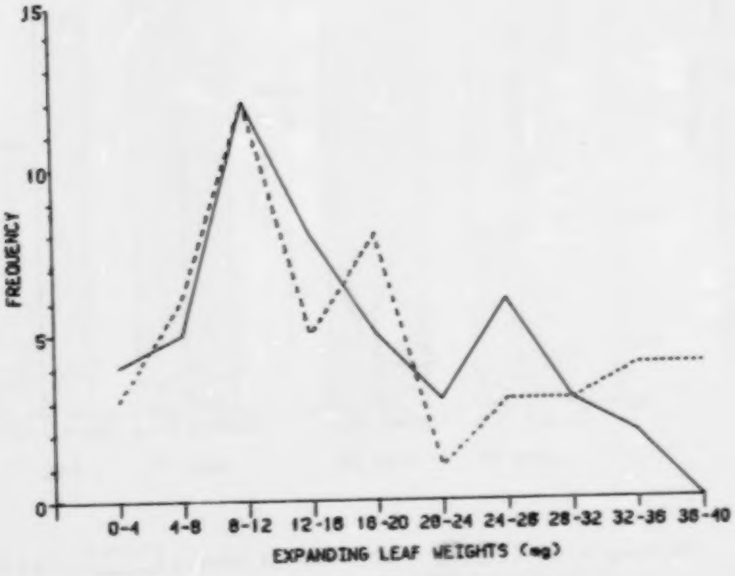

Figure 4.1. Weight distribution of expanding leaves of *Agropyron smithii* plants on the Control (———) and SO_2 (- - - -) treatment.

and leaf age class (P = 0.03). Plants receiving SO_2 displayed significantly higher belowground ^{14}C concentrations on June 18 both compared to the Control plants and to other dates within the SO_2 treatment (Figure 4.2). Above- to belowground ^{14}C concentrations within the Control were not influenced by date. The significant date and treatment effects can be attributed to the June 18 high rhizome ^{14}C concentration in plants on the SO_2 treatment (Table 4.2).

The age class of the labeled leaf influenced above- to belowground ^{14}C concentrations in plants on the SO_2 treatment but not in Control plants (Figure 4.3). Belowground ^{14}C concentration on the SO_2 treatment became proportionately less when younger leaves higher on the grass culm were labeled. This occurred in response to the younger and older leaves respective proximity to greater sink demand of the expanding leaves and the rhizomes of plants on the SO_2 treatment. However, even in the presence of greater aboveground assimilate demand in plants on the SO_2 treatment, the concentrations of belowground ^{14}C between plants on the Control and SO_2 treatments were not different when the youngest leaves were labeled and were greater on the SO_2 treatment when the older leaves were labeled.

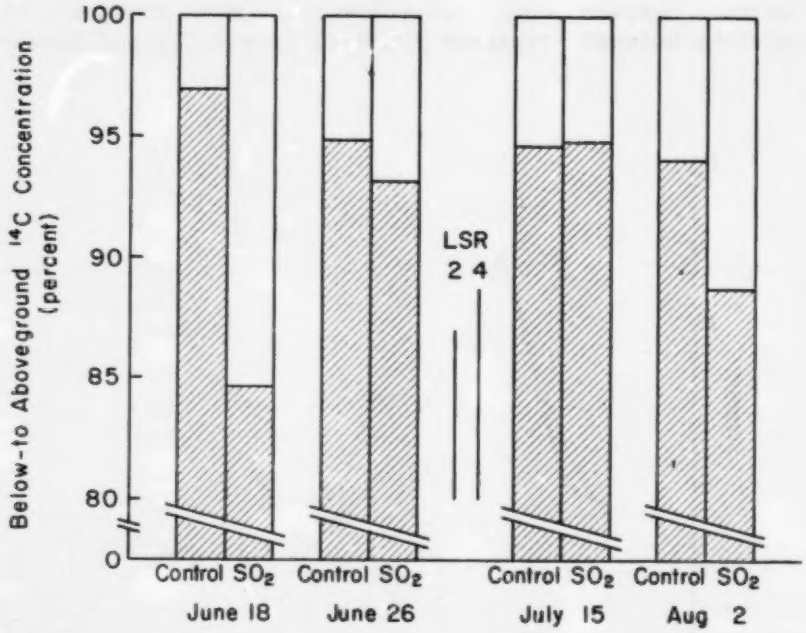

Figure 4.2. Aboveground (▨) to belowground (☐) relative ^{14}C concentrations (CPM/mg) for Control and SO_2 treatments by date. Use LSR_2 for within date across treatment comparisons; and LSR_4 for within treatment across date comparisons.

TABLE 4.2. CARBON-14 RELATIVE CONCENTRATION IN BELOWGROUND COMPONENTS. BELOWGROUND PARTITIONING DATA IS NOT PRESENTED BECAUSE 100 PERCENT OF THE BELOWGROUND BIOMASS WAS NOT SAMPLED

			^{14}C Mean concentration (%)		
Date	Treatment	Plant Part	Labeled Old Leaf CPM/mg	Labeled Middle Leaf CPM/mg	Labeled Young Leaf CPM/mg
June 18	Control	Root	0.9	0.9	0.5
		Rhizome	0.6	5.2	0.7
	SO_2	Root	9.2	3.0	3.1
		Rhizome	17.5	11.9	1.5
June 26	Control	Root	1.4	3.4	2.4
		Rhizome	2.3	2.7	3.1
	SO_2	Root	4.1	4.2	2.4
		Rhizome	3.3	2.7	4.2
July 15	Control	Root	1.6	2.7	2.6
		Rhizome	1.7	3.5	4.0
	SO_2	Root	3.9	2.3	2.9
		Rhizome	3.2		1.1
August 2	Control	Root	1.1	1.0	1.0
		Rhizome	4.2	3.9	6.6
	SO_2	Root	3.9	4.3	2.6
		Rhizome	6.7	10.3	6.1

DISCUSSION

A stimulation in translocation of ^{14}C from the labeled leaf in the aboveground compartment was observed in *Agropyron smithii* exposed on the ZAPS I High plot. The increased translocation was in response to the greater sink demand of developing leaves. Export to the developing leaf was greatest from the young fully expanded leaf nearest the sink. The greater sink demand of developing leaves in the plants exposed to SO_2 could be the result of more rapid cell division and growth rates, or because a slower development of photosynthetic capacity in the developing leaves exposed to SO_2 requires greater or longer carbon import from mature leaves before they are self sufficient. Increased translocation from mature leaves which are fixing less carbon is possible because sink demand can override the availability of

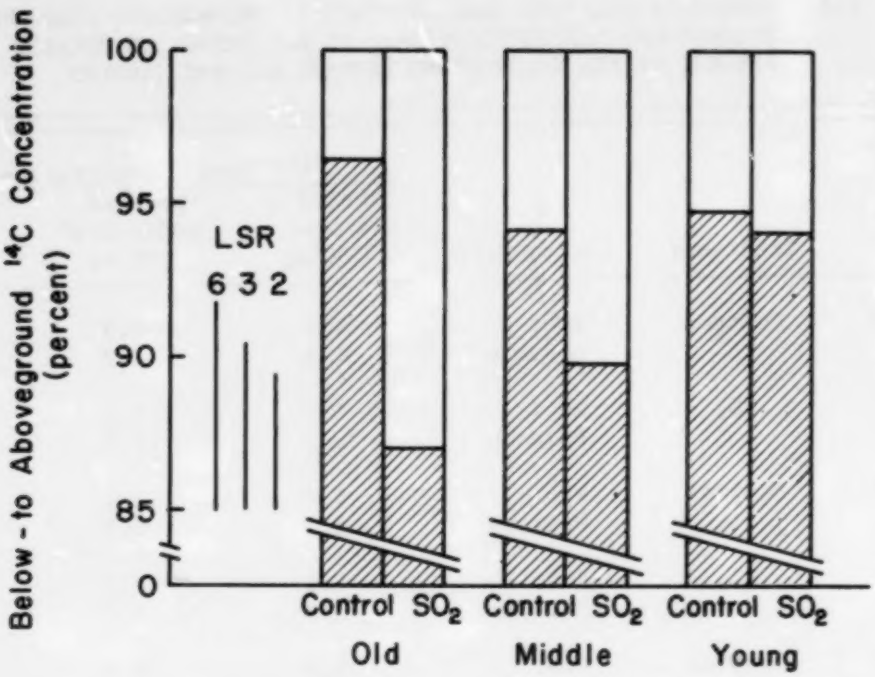

Figure 4.3. Aboveground (▨) to belowground (□) relative ^{14}C concentrations (CPM/mg) for Control and SO_2 treatments when the old, middle and young leaves were labeled. Use LSR$_2$ for within leaf age class across treatment comparisons, LSR$_3$ for within treatment across leaf age class comparisons, and LSR$_6$ for across treatment across leaf age class comparisons.

assimilate in regulating translocation (Ho, 1979). It is first necessary to assess plant growth and photosynthetic rate in response to SO_2 concentrations before a hypothesis can be formulated concerning the increased translocation with SO_2 exposure observed in this experiment.

Photosynthetic rates are inhibited at high SO_2 concentrations (Bennett and Hill, 1974; White *et al.*, 1974; Sij and Swanson, 1974; Koziol and Jordan, 1978). However, some data suggest a stimulation at low SO_2 concentrations (Muller *et al.*, 1979; Katz *et al.*, 1939; Thomas and Hill, 1937). Low SO_2 concentrations apparently stimulate the Hill-reaction and benefit overall photosynthesis whereas at higher concentration RuDP-carboxylase is inhibited (Ziegler, 1972). Thor (1980), in an extensive review of the SO_2 concentration and photosynthetic response literature, estimated a stimulation in photosynthesis may occur up to SO_2 concentrations of about 20 pphm. Measurements of the photosynthetic rate of *Agropyron smithii* on our study area during the period of this experiment showed no trend of either stimulation or inhibition (Thor, 1980). However, in the drier previous year 39 percent stimulation in the photosynthetic rate of *Agropyron smithii* was detected on the SO_2 treatment.

A significant SO_2 effect on net primary or *Agropyron smithii* productivity could not be detected on our study area (Dodd *et al.*, In Prep.). However measuring individual leaf areas of *Agropyron smithii*, Milchunas *et al.* (In Prep.) observed a significant increase in leaf areas on Zaps II Low SO_2 exposure but only a trend of increased leaf area with the High treatment examined in this study. It was concluded that the Low treatment was optimum for stimulating growth under the abiotic and nutrient conditions on our study area, but that the High treatment had detrimental effects which were operative but not at a level that fully negated the fertilizer effect.

Two points are apparent from the productivity and photosynthetic responses in conjunction with ^{14}C translocation. First, the increased ^{14}C translocation with SO_2 exposure observed in this study is in response to stimulated growth rates. Second, productivity and photosynthetic rate measurements are not as sensitive as translocation to low level SO_2 exposure. Translocation was also observed to be more sensitive to SO_2 than photosynthesis by Teh and Swanson (1977) for a very high (300 pphm) SO_2 concentration, and by Noyes (1980) for concentrations of 10, 100 and 300 pphm. They reported that translocation was inhibited more than photosynthesis. The inhibition of translocation with 10 pphm SO_2 reported by Noyes is not consistent with the increased translocation we observed on the High treatment. This may be attributed to several major differences between experimental designs of the two experiments. Noyes used 11-day-old hydroponically growing bean plants with one remaining leaf on the plant at the time of labeling. Translocation was monitored after the leaves were exposed to SO_2 for 2 hours. This was in contrast to our continued SO_2 exposure of field growing plants with the influence of intact aboveground sinks. Plant species, environmental conditions, SO_2 dose duration and concentration fluctuation with possible plant adaptation, and sink demands may have contributed to the different responses observed between the two studies. Noyes (1980) suggested SO_2 inhibited sieve-tube loading. If this occurs, the results from this study suggest that within a fertilizing range of low SO_2 dose, stimulated sink strength can override the mechanism involved in sieve-tube loading inhibition.

The concentration of translocated ^{14}C may be more sensitive to the effects of SO_2 than harvest data or photosynthetic measurements both from a technique standpoint and from a standpoint of measuring specific growth rate. Population productivity measurements by field clip plots are subject to high variability because of differences in species density and composition. Subtle responses may be less than sampling variance. Growth increments measured with the individual plant as the unit of measure are diluted by a factor of the variable nongrowing structural biomass. When growth is occurring in a particular organ, the resolution of measurements of growth rate declines as measurements proceed from the organ to the individual to the population. Photosynthesis is a measure of assimilation and not necessarily utilization. The effects of SO_2 manifest in biochemical pathway and functional mechanisms after the assimilation process. In the absence of growth, assimilation may proceed at a maintenance level whereas translocation is negligible in a sinkless system. During growth, translocation is not entirely a function of internal concentration gradients but is also hormonally regulated. Dividing cells produce auxins which further stimulates growth but also stimulates phloem transport (Davies and Wareing, 1965; Patrick and Wolley, 1973; Seth and Wareing, 1967; Patrick, 1976). The concentration of translocated ^{14}C is thus a sensitive index of growth rate because of specificity to the particular organ involved in growth.

Differences in the concentration and partitioning of ^{14}C between Control and SO_2 exposed plants were greatest for the expanding leaf. Although concentrations of ^{14}C in the expanding leaf were often high, this organ received only a minor portion of the ^{14}C pool. Even in plants exposed to SO_2, only 4 percent of the total aboveground ^{14}C was located in the expanding leaf. Many workers have stressed the lack of movement of assimilate into any but actively growing young organs or developing reproductive parts. In this study, ^{14}C was detected in mature leaves but accounted for only 0.5 and 1.0 percent of the total aboveground ^{14}C pool for Control and SO_2 treatments, respectively. It is not known, however, whether the ^{14}C entering the mature leaves was in sugars, or in amino acids synthesized in the roots and redistributed. By far the largest pool of exported ^{14}C was found in the "other" plant parts (Table 4.1) where 17 and 22 percent of the aboveground ^{14}C was partitioned in the Control and SO_2 treatment plants, respectively. Considering that 1) the "other" category consisted of the stem and mature leaves excluding the top three leaves, and 2) that mature leaves imported very small quantities of ^{14}C, the majority of exported ^{14}C was located in the stem.

Exposure of *Agropyron smithii* on the High treatment increased ^{14}C concentrations in the belowground compartment only on the first sampling date. Our data suggest that this was in response to a greater rhizome sink demand. The initiation of tillers is probably similar to that of developing leaves and requires import of carbon until a self contained photosynthetic capacity is attained. Coughenour *et al.* (1979) labeled *Agropyron smithii* plant with ^{14}C on the same study area but in a previous year. They observed stimulated root growth on the SO_2 treatment in July. The observation of increased translocation to aboveground sinks in plants exposed to SO_2 concurrent with an increase in relative belowground ^{14}C concentrations indicates a stimulation in reproductive root and vegetative growth on the High treatment.

Data from primary producer studies during the five years of fumigating this particular grassland may at first seem contradictory. We have observed SO_2 stimulated translocation, photosynthesis (Thor, 1980), and leaf areas (Milchunas *et al.*, In Prep.) concurrent with high sulfur accumulation (Lauenroth *et al.*, 1979; Milchunas *et al.*, 1980), increased rates of senescence (Heitschmidt *et al.*, 1978, Milchunas *et al.*, In Prep.) and reductions in chlorophyll (Lauenroth and Dodd, 1980) with no measurable effect of SO_2 on standing biomass (Dodd *et al.*, In Prep.). Other researchers have observed protein hydrolysis and the accumulation of free amino acids in plants exposed to SO_2 (Godzik and Linskens, 1974; Malhotra and Sarkar, 1979; Constantinidou and Kozlowski, 1979). These seemingly contradictory findings can be synthesized into a conceptual model explaining one aspect of how SO_2 influences a plant's life history if we differentiate between the overall response of the plant as an individual or population and the organ or biochemical components and compare them with the known sequence of events in the life of an unperturbed plant. The hypothesis presented considers relatively low to moderate SO_2 concentrations rather than levels where immediate and acute toxic responses occur.

The growth and senescence of plants is a complex process of nutrient and carbon assimilation, metabolism, and distribution. Sulfur is a required plant nutrient and atmospheric SO_2 is one source of sulfur. Thus, SO_2 is both a fertilizer and a toxic gas depending on the duration and concentration of exposure and the plant's nutrient status. Below optimum nitrogen to sulfur ratios were corrected in *Agropyron smithii* plants exposed to SO_2 (Milchunas *et al.*, 1980). A stimulation in growth would then be expected and increased leaf areas with SO_2 exposures confirms this (Milchunas *et al.*, In Prep.). Although leaves grew at a faster rate, they also senesced at a more rapid rate. An increased rate of senescence has been one of the commonly reported responses to SO_2 exposure (Garsed *et al.*, 1979; Bleasdale, 1973; Bell and Clough, 1973; Matsushima and Harada, 1966; Heitschmidt *et al.*, 1978; Milchunas *et al.*, In Prep.). This same phenomena occurred with the application of nitrogen fertilizer (Milchunas *et al.*, In Prep.).

The question then arises as to whether the impact of SO_2 at low levels is due to toxicity or a nutrient input perturbation which accelerates the growth and senescence process. Reductions in chlorophyll and protein hydrolysis occur in the natural sequence of plant senescence (Scott and Leopold, 1966). As leaf tissue enters into senescence, it experiences an extensive hydrolysis of protein components (dela Fuente and Leopold, 1968; Osborne, 1973; Abeles *et al.*, 1967). A wide array of amino acids accumulate as a consequence of the protein hydrolysis, including especially glutamine (Plaisted, 1958). With regard to the amino acid metabolism of plants exposed to SO_2, the most important changes seem to be the increase of glutamine, NH_3, asparagine, and alanine (Godzik and Linskens, 1974). Michael (1936) established that the progress of leaf senescence was facilitated when soluble amide products could be translocated out of the leaf tissues. Further, alanine brings about abundant ethylene production and ethylene is important in the induction of senescence and leaf abscission (Abeles, 1967; dela Fuente and Leopold, 1968). Data of Williams (1955) suggested that rapid shoot growth could accelerate the senescence of more mature tissues by inducing the hydrolysis of nucleic acids. This is supported by the observation that regions of the plant that are rich in auxin (the young expanding parts) (Burg and Burg, 1968) are also regions of high ethylene production (Osborne, 1973). Young plant parts are not, however, susceptable to the ethylene (Abeles, 1967). This provides a feedback mechanism for transport of nutrients from older leaves to younger leaves. The role of auxin as a mobilizer of nutrients has been well established (Addicott, 1970).

The following is a hypothetical model of a mechanism for low-concentration SO_2 perturbation in the absence of visible and acute injury. In early spring, low concentrations of SO_2 supplement soil sulfur necessary for optimum nitrogen to sulfur ratios. Sulfur dioxide entry through leaf stomata compensates for differential absorption or translocation rates of N and S. A fertilizing effect ensues which stimulates growth. Stimulated growth in new leaves is a strong sink which increases translocation from older leaves. The older leaves have now been exposed to SO_2 for a long period of time. The sulfur concentration that has built up is relatively immobile and does not leave the leaves as readily as does nitrogen (Williams, 1955). The build up of sulfur causes a loss of membrane integrity releasing SF (senescence factor) which enhances ethylene biosynthesis. The older leaves are also susceptible to the ethylene produced in the young expanding leaves. Protein hydrolysis and the mobilization of nutrients and chlorophyll to younger plant parts ensues. In this manner stimulated growth and translocation, protein hydrolysis, an increased rate of senescence, reduced chlorophyll content, with no change in total standing biomass can all be explained. Increased translocation rates can be a function of the more rapid turnover and the mobilization of nutrients as senescence proceeds.

There appears then to be good correlation between the natural senescence processes in plants and the responses to SO_2. A relationship between sulfur content and date of abscission in leaves of *Quercus rubra* has been observed (Garsed *et al.*, 1979). Premature leaf fall seems to be a common response of both coniferous and deciduous trees in industrial areas, and certain trees that are normally evergreen may be come deciduous in some polluted regions (Scurfield, 1960).

CONCLUSIONS

Exposure of *Agropyron smithii* Rybd. on the High SO_2 treatment increased translocation of photoassimilated $^{14}CO_2$ to rhizomes early in the growing season and to developing leaves throughout the growing season. Leaves near the top of the plant supplied proportionately more assimilate to the developing leaf while lower leaves partitioned a greater proportion to a SO_2 stimulated rhizome sink. Examination of productivity and photosynthesis data concurrent with translocation responses suggested that subtle effects of low-level SO_2 exposure may best be monitored through ^{14}C partitioning studies.

REFERENCES

Abeles, F. B. 1967. Mechanism of Action of Abscission Accelerators. Physiol. Plant., 20:442-454.

Abeles, F. B., R. E. Holm, and H. E. Gahagan. 1967. Abscission: The Role of Aging. Plant Physiol., 42:1351-1356.

Addicott, F. T. 1970. Plant Hormones in the Control of Abscission. Biol. Rev., 45:485-524.

Bell, J. N. B., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulphur Dioxide. Nature, 241:47-49.

Bennett, J. H., and A. C. Hill. 1974. Acute Inhibition of Apparent Photosynthesis by Phytotoxic Air Pollutants. In: Air Pollution Effects on Plant Growth. Mark Dugger, ed. American Chemical Society, Washington, D.C. 150 p.

Bleasdale, J. K. A. 1973. Effects of Coal-smoke Pollution Gases on the Growth of Ryegrass (*Lolium perenne* L.). Environ. Pollut., 5:275-285.

Burg, S. P., and E. A. Burg. 1968. Auxin Stimulated Ethylene Formation. Its Relationship to Auxin Inhibited Growth, Root Geotropism and Other Plant Processes. In: Biochemistry and Physiology of Plant Growth Substances. F. Wightman and G. Setterfield, eds. Runge Press, Ottawa. pp. 1275-1294.

Constantinidou, H. A., and T. T. Kozlowski. 1979. Effects of Sulfur Dioxide and Ozone on *Ulmus americana* Seedlings. II. Carbohydrates, Proteins, and Lipids. Can. J. Bot., 57:176-184.

Coughenour, M. B., J. L. Dodd, D. C. Coleman, and W. H. Lauenroth. 1979. Partitioning of Carbon and SO_2-sulfur in a Native Grassland. Oecologia, 42:229-240.

dela Fuente, R. K., and A. C. Leopold. 1968. Senescence Processes in Leaf Abscission. Plant Physiol., 43:1496-1502.

Davies, C. R., and P. F. Wareing. 1965. Auxin Induced Transport of Radiophosphorus in Stems. Planta, 65:139-156.

Dodd, J. L., W. K. Lauenroth, and R. K. Heitschmidt. In Prep. Effects of Controlled SO_2 Exposure on Net Primary Production and Plant Biomass Dynamics. In: Bioenvironmental Impact of a Coal-fired Power Plant, Sixth Interim Report, Colstrip, Montana. E. M. Preston, D. W. O'Guinn, and R. A. Wilson (eds.) Environmental Protection Agency, Corvallis, Oregon.

Fellows, R. J., and D. R. Geiger. 1974. Structural and Physiological Changes in Sugar Beet Leaves During Sink to Source Conversion. Plant Physiol., 54:877-885.

Garsed, S. G., J. F. Farrar, and A. J. Rutter. 1979. The Effect of Low Concentrations of Sulphur Dioxide on the Growth of Four Broadleaved Tree Species. J. Appl. Ecol., 16:217-226.

Geiger, D. R. 1979. Control of Partitioning and Export of Carbon in Leaves of Higher Plants. Bot. Gaz., 140:241-248.

Godzik, S., and H. F. Linskens. 1974. Concentration Changes of Free Amino Acids in Primary Bean Leaves after Continuous and Interrupted SO_2 Fumigation and Recovery. Environ. Pollut., 7:25-38.

Heitschmidt, R. K., W. K. Lauenroth, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulphur Dioxide on Western Wheatgrass in a Southeastern Montana Grassland. J. Appl. Ecol., 14:859-868.

Ho, L. C. 1979. Regulation of Assimilate Translocation between Leaves and Fruits in the Tomato. Ann. Bot., 43:437-448.

Kamanina, M. S., and A. A. Anisimov. 1977. Escape of Assimilates From the Mesophyll into the Void Space of Leaves Under Different Conditions of Mineral Nutrition. Soviet Plant Physiol., 24:628-632.

Katz, M., G. A. Ledingham, and A. E. Harris. 1939. Carbon Dioxide Assimilation and Respiration of Alfalfa Under Influence of Sulphur Dioxide. In: Effect of Sulfur Dioxide on Vegetation. The Associate Committee on Trail Smelter Smoke of the National Research Council of Canada. 206 p.

Koziol, M. J., and C. F. Jordan. 1978. Changes in Carbohydrate Levels in Red Kidney Bean (*Phaseolus vulgaris* L.) Exposed to Sulphur Dioxide. J. Exp. Bot., 29:1037-1043.

Lauenroth, W. K., C. J. Bicak, and J. L. Dodd. 1979. Sulfur Accumulation in Western Wheatgrass Exposed to Three Controlled SO_2 Concentrations. Plant and Soil, 53:131-136.

Lauenroth, W. K., and J. L. Dodd. 1980. Chlorophyll Reduction in Western Wheatgrass Exposed to Sulfur Dioxide. In: Bioenvironmental Impact of a Coal-fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. O'Guinn (eds.) EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 197-203.

Malhotra, S. S., and S. K. Sarkar. 1979. Effects of Sulphur Dioxide on Sugar and Free Amino Acid Content of Pine Seedlings. Physiol. Plant., 47:223-228.

Matsushima, J., and M. Harada. 1966. Sulfur Dioxide Gas Injury to Fruit Trees. V. Absorption of Sulfur Dioxide by Citrus Trees and its Relation to Leaf Fall and Mineral Contents of Leaves. J. Jap. Soc. Hort. Sci., 35:242-246.

Michael, G. 1936. Über die Deziehungen zwischen chlorophyll und Eiweissabbau im virgilbenden Laubblatt von *Tropoeolum*. Z. Botan., 29:385-425.

Milchunas, D. G., W. K. Lauenroth, and J. L. Dodd. In Prep. Effects of SO_2 Exposure with Nitrogen and Sulfur Fertilization on the Growth of *Agropyron smithii* Rybd. In: Bioenvironmental Impact of a Coal-fired Power Plant, Sixth Interim Report, Colstrip, Montana. E. M. Preston, D. W. O'Guinn, and R. A. Wilson (eds.) Environmental Protection Agency, Corvallis, Oregon.

Milchunas, D. G., W. K. Lauenroth, and J. L. Dodd. 1980. Forage Quality of Western Wheatgrass Exposed to Sulfur Dioxide. J. Range Manage.

Mor, Y., and A. H. Halevy. 1979. Translocation of ^{14}C-assimilates in Roses. I. The Effect of the Age of the Shoot and the Location of the Source Leaf. Physiol. Plant., 45:177-182.

Muller, R. N., J. E. Miller, and D. G. Sprugel. 1979. Photosynthetic Response of Field-grown Soybeans to Fumigations With Sulphur Dioxide. J. Appl. Ecol., 16:567-576.

Noyes, R. D. 1980. The Comparative Effects of Sulfur Dioxide on Photosynthesis and Translocation in Bean. Physiol. Plant Pathol., 16:73-79.

Osborne, D. J. 1973. Internal Factors Regulating Abscission. In: Shedding of Plant Parts, T. T. Kozlowski, ed. Academic Press, New York. pp. 125-147.

Patrick, J. W. 1976. Hormone-directed Transport of Metabolites. In: Transport and Transfer Processes in Plants. I. F. Wardlaw and J. B. Passioura, eds. Academic Press, New York. Chapter 37, p. 433-446.

Patrick, J. W., and D. J. Wolley. 1973. Auxin Physiology of Decapitated Stems of *Phaseolus volgaris* L. Treated With Indol-3yl-acetic Acid. J. Exp. Bot., 24:949-957.

Plaisted, P. H. 1958. Some Biochemical Changes During Development and Aging of *Acer platanoides* L. Leaves. Contribs. Boyce Thompson Inst., 19:245-254.

Scott, P. C., and A. C. Leopold. 1966. Abscission as a Mobilization Phenomenon. Plant Physiol., 11:826-830.

Scurfield, G. 1960. Air Pollution and Tree Growth. Forestry Abstr., 21:339-347, 517-528.

Seth, A. R., and P. F. Wareing. 1967. Hormone-directed Transport of Metabolites and Its Possible Role in Plant Senescence. J. Exp. Bot., 18:65-77.

Sij, J. W., and C. A. Swanson. 1974. Short-term Kinetic Studies on the Inhibition of Photosynthesis by Sulfur Dioxide. J. Environ. Qual., 3: 103-107.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. Wit Freeman and Co., San Francisco. 776 pp.

Swanson, C. A., J. Hoddinott, and J. W. Sij. 1976. The Effect of Selected Sink Leaf Parameters on Translocation Rates. In: Transport and Transfer Processes in Plants. I. F. Wardlaw and J. B. Passioura, eds. Academic Press, New York. p. 347-356.

Teh, K. H., and C. A. Swanson. 1977. Sulfur Dioxide Inhibition of Translocation and Photosynthesis in *Phaseolus vulgaris* L. Plant Physiol., (Bethesda) 59 (6 Suppl.):123.

Thomas, M. D., and G. R. Hill. 1937. Relation of Sulphur Dioxide in the Atmosphere to Photosynthesis and Respiration of Alfalfa. Plant Physiol., 12:309-383.

Thor, G. L. 1980. Photosynthesis in Western Wheatgrass Exposed to Chronic SO_2 Air Pollution. M. S. Thesis, Colorado State University, Ft. Collins.

Thrower, S. L. 1962. Translocation of Labelled Assimilates in Soybeans. II. The Pattern of Translocation in Intact and Defoliated Plants. Aust. J. Biol. Sci., 15:629-649.

Wardlaw, I. F. 1968. The Control and Pattern of Movement of Carbohydrates in Plants. Bot. Rev., 34:79-105.

White, K. L., A. C. Hill, and J. H. Bennett. 1974. Synergistic Inhibition of Apparent Photosynthesis Rate of Alfalfa by Combinations of Sulfur Dioxide and Nitrogen Dioxide. Environ. Sci. and Technol., 8:574-576.

Williams, R. F. 1955. Redistribution of Mineral Elements During Development. Ann. Rev. Plant. Physiol., 6:25-42.

Ziegler, I. 1972. The Effect of $SO_3^=$ on the Activity of Ribulose-1, 5-Diphosphate Carboxylase in Isolated Spinach Chloroplasts. Planta, 103: 155-163.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79-105.

SECTION 5

EFFECTS OF LOW-LEVEL SULFUR DIOXIDE EXPOSURE ON DECOMPOSITION OF WESTERN WHEATGRASS (*AGROPYRON SMITHII*) LITTER UNDER LABORATORY CONDITIONS

J. W. Leetham, J. L. Dodd, and W. K. Lauenroth

ABSTRACT

A laboratory study of the effects of chronic low-level SO_2 exposure on decomposition of western wheatgrass (*Agropyron smithii*) was conducted. Finely ground western wheatgrass litter was exposed to 8.5 pphm SO_2 continuously for 5 weeks. Samples were periodically measured for respiration and total decomposition. Results showed a significant 9-17 percent reduction in decomposition rates under SO_2 exposure. Respiration rate differences were not detectable with the techniques used. It is speculated that lowered pH conditions in the litter and/or the accumulation of toxic SO_2 derivatives were responsible for reduced microbial activity.

INTRODUCTION

Soil surface decomposition processes are responsible for the breakdown of a majority of aboveground herbage biomass in natural grasslands. Surface decomposition is essential for the release of organic products into the subsurface nutrient cycling processes that form the basic support of the whole ecosystem. Any major disturbance of the decomposition processes will ultimately affect all other components of the system. A major disturbance can be a large-scale, short-term disturbance, or a low-level, chronic disturbance which may take considerable time to bring about major changes in the system.

Babich and Stotzky (1974) have reviewed the literature dealing with air pollutant interactions with microorganisms and cite numerous studies of the toxicity of SO_2, especially with increasing soil acidity. Bååth *et al.* (In Press) and Abrahamsen *et al.* (1978) in Sweden and Norway respectively have shown that artificial acidification of coniferous forest soils significantly reduced soil microbial biomass and activity and overall decomposition of leaf litter. Grant *et al.* (1979) found reduced microbial activity in acidic forest soil when it was exposed to 100 pphm SO_2. Saunders (1973) reviewed the effects of air pollutants on leaf surface microflora and cites much evidence of deleterious effects of SO_2 on the microorganisms. Wodzinski and Alexander (1978) provide evidence of significant effects of SO_2 on algal photosynthetic rates. There is considerable usage of SO_2 in the food production and preservation industry to completely inhibit various types of microbial activity.

Dodd and Lauenroth (1979) reported up to 43 percent reductions in decomposition rates in western wheatgrass leaves with chronic low-level SO_2 exposure under field conditions. It was that finding which precipitated this study for the purpose of verifying the field data with more precise laboratory control.

MATERIALS AND METHODS

The exposure system used in this study was relatively simple in design. It consisted of a plexiglass chamber measuring 0.61 m x 0.61 m x 1.83 m (2 ft x 2 ft x 6 ft). Air movement through the chamber was a single pass, push-pull system which created a slight negative internal pressure. Source SO_2 was released from a temperature-stabilized bottle of liquid SO_2 directly into the inlet air stream to create the desired SO_2 concentration. An identical chamber without SO_2 was used for the control. The chambers were housed in a room maintained at 24°C. Details of the chamber construction and operation are described by Leetham *et al.* (1980).

Sulfur dioxide concentration in the treatment chamber was maintained at 8.5 pphm as measured by a flame photometric sulfur gas analyzer (Meloy Laboratories Inc., Model SA-160) at the exhaust fan orifice. Because of slight variability of SO_2 concentration within the chamber, other measurements were made at various locations on the shelves where the litter samples were maintained. Temporal variation in SO_2 concentration was less than ±1 pphm and spatial variation did not exceed ±2 pphm for all points measured.

Test material was predominantly western wheatgrass (*Agropyron smithii* Rydb.) litter gathered from a native northern mixed-grass prairie site in southeastern Montana in May, 1979. The litter was dead material grown during the previous (1978) growing season and had not been previously exposed to any major air pollutants. The litter was finely ground in a Willey mill and thoroughly mixed to a homogenous state. Ash content was measured by ashing in a muffle furnace at 600°C. Mean ash content was 12.36 percent (S.E. = 0.17 percent) for 10 subsamples of the homogenate. To maintain the natural microorganism inoculate, the litter was dried at 30°C prior to weighing out test parcels. Water content of the litter at 30°C was 5.15 percent (S.E. = 0.02 percent, n = 10) and was accounted for in weight loss calculations.

A schematic of the test sample dish is provided in Figure 5.1. Each sample consisted of a 2 g layer of litter over a 20 g layer of washed and autoclaved sand in a 60 x 15 mm plastic petri dish. The washed sand was determined to have a mean ash-free organic content of 0.46 percent (S.E. = 0.02 percent, n = 10). A small access port was made through the litter layer in the center of the dish to allow periodic watering by trickling distilled water through the access port into the sand. The litter was moistened by absorption of water from the sand rather than by surface flooding. Since high relative humidity was not maintained in chambers, evaporation required daily addition of

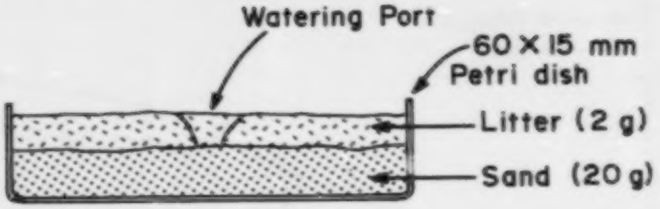

Figure 5.1. Schematic of sample dish for decomposition study.

approximately 5 ml of water per sample. Dishes containing only sand were maintained the same as those with litter.

The experiment was initiated on 6 September 1979 when all litter samples were placed in the control and SO_2 treatment chambers. Samples were randomly selected and removed for measurements on each of four sampling dates (17, 24 September and 1, 8 October). Sample size was 10 per chamber on the first three dates and 20 per chamber on the final date. After removal from the chambers the samples were watered, as during the incubation period, and respiration rates were measured over a 4 hour test period with the alkali absorption technique described by Coleman *et al.* (1978). The samples were then oven-dried at 60°C and weighed. Decomposition was expressed as percentage loss of original ash-free dry weight. Daily loss rates were calculated by dividing ash-free weight-loss for a given interval by ash-free weight at the beginning of the interval and by the duration (days) of the interval.

RESULTS AND DISCUSSION

Significant respiration rate differences between treated and control samples were not detectable. For example, on the second sample date the average respiration rates were 0.2531 $\mu g\ CO_2 \cdot Sample^{-1} \cdot min^{-1}$ (S.E. = 0.0084) and 0.2512 $\mu g\ CO_2 \cdot Sample^{-1} \cdot min^{-1}$ (S.E. = 0.0058) for the control and SO_2 treatment respectively. No consistent trend was observed across time. No respiration was detected in the dishes containing only sand.

An obvious decrease in litter decomposition in the SO_2-exposed dishes was observed on all sample dates (Figure 5.2). A split-plot design ANOVA was performed on the data to test for treatment and time differences. Treatment differences were significant at $P = .075$ and the date differences were significant at $P < .001$. Standard errors of the means for any date-treatment ranged from 2 to 4 percent of the means.

Decomposition rates for both the control and SO_2 treated samples were greater during the first 11 days of the test (11.5 and 10.5 $\mu g \cdot g^{-1} \cdot d^{-1}$, respectively) than for the last 21 days of the test (4.2 and 3.5 $\mu g \cdot g^{-1} \cdot d^{-1}$, respectively). This demonstrates increasing resistance of the litter material to decay with time, a characteristic pattern in decomposition of relatively fresh plant litter (Christie, 1979; Howard and Howard, 1974) which

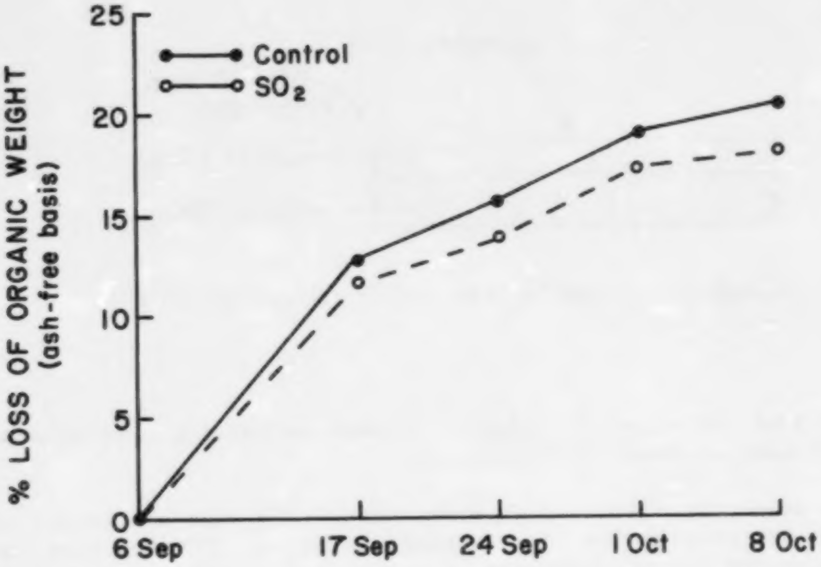

Figure 5.2. Decomposition in control and SO_2 exposed western wheatgrass litter over four sample dates. Figures are average total weight loss expressed as a percentage of original starting weight (less residual water content).

is a consequence of rapid initial decay of labile compounds such as sugars, starches, and proteins, followed by much slower decay of more resistant compounds (cellulose, lignin, fats, tannins, waxes) (Hunt, 1977).

Inhibition of decomposition rates by SO_2 was less during the first interval than in the last two-thirds of the test period (9 versus 17 percent reduction in daily loss rate). This strongly suggests a cumulative effect of exposure to SO_2, since atmospheric SO_2 concentrations were constant, and is probably due to the accumulation of toxic SO_2 derivatives such as sulfate or sulfite or a reduction in pH of the litter-microbe system.

CONCLUSIONS

Although the significance level of the treatment differences ($P = 0.075$) exceeded the "traditional" 0.05 level of acceptance, we are concluding they are real since the trend is strong and consistent with time and the results corroborate the findings of Dodd and Lauenroth (1979). They reported 12-43 percent reductions in decomposition rates in response to season-long exposures of western wheatgrass leaves to low-level SO_2 under field conditions. These studies present strong evidence that chronic exposure to SO_2 in low concentrations can cause a significant reduction in microbial activity in soil surface litter. Neither study attempted to identify the mechanisms of inhibition of microbial activity. We suspect that a reduction in pH in the litter and/or the accumulation of toxic SO_2 derivatives to be the probable mode of action of SO_2 on the microorganisms. Other studies have found reductions in acidity do reduce microbial activity (Abrahamsen *et al*, 1978; Bååth *et al*., 1980; Saunders, 1973; Wodzinski and Alexander, 1978; and Grant *et al*., 1979).

The asymptotic curve of decomposition over time (Figure 5.2) was expected because the mechanical grinding of the old, partially decomposed litter exposed more labile material previously unavailable to the microorganisms and they quickly acted upon it. Had the study been carried out over a longer period of time, the curve undoubtedly would have flattened out even more, but we suspect treatment differences would have been magnified.

Respiration rate differences were not detected probably because of the technique used. We suspect the respiration rates were different since respiration is a good index to microbial activity and we are concluding that the microbial activity was reduced by SO_2. More refined techniques of respiration measurement would be needed to accurately measure the small differences that probably occurred in our experimental design.

REFERENCES

Abrahamsen, G., J. Hovland, and S. Hågvar. 1978. Effects of Artificial Acid Rain and Liming on Soil Organisms and the Decomposition of Organic Matter. SNSF-contribution FA 28/78. 23 pp.

Bååth, E., B. Berg, U. Lohm, B. Lundgren, H. Lundkvist, T. Rosswall, B. Söderstrom, and A. Wiren. 1980. Effects of Experimental Acidification and Liming on Soil Organisms and Decomposition in a Scots Pine Forest. Pedobiologia (in press).

Babich, H., and G. Stotzky. 1974. Air Pollution and Microbial Ecology. Crit. Rev. Environ. Control, 4:353-421.

Christie, E. K. 1979. Ecosystem Processes in Semi-arid Grasslands. II. Litter Production, Decomposition, and Nutrient Dynamics. Aust. J. Agric. Res., 30:29-42.

Coleman, D. C., R. V. Anderson, C. V. Cole, E. T. Elliott, L. Woods, and M. K. Campion. 1978. Trophic Interactions in Soils as they Affect Energy and Nutrient Dynamics. IV. Flows of Metabolic and Biomass Carbon. Microbial Ecology, 4:373-380.

Dodd, J. L., and W. K. Lauenroth. 1980. Effects of Low-level SO_2 Fumigation on Decomposition of Western Wheatgrass Litter in a Mixed-Grass Prairie. In: Bioenvironmental Impact of a Coal-fired Power Plant. Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn (eds.) EPA-600/3-80-052. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 212-215. (In Press).

Grant, I. F., K. Bancroft, and M. Alexander. 1979. SO_2 and NO_2 Effects on Microbial Activity in an Acid Forest Soil. Microbial Ecology, 5:85-89.

Howard, P. J. A. and D. M. Howard. 1974. Microbial Decomposition of Tree and Shrub Leaf Litter. I. Weight Loss and Chemical Composition of Decomposing Litter. Oikos, 25:341-352.

Hunt, H. W. 1977. A Simulation Model for Decomposition in Grasslands. Ecology, 58:469-484.

Leetham, J. W., W. Ferguson, J. L. Dodd, and W. K. Lauenroth. 1980. Design and Construction of a Simple, Continuous Flow Sulfur Dioxide Exposure Chamber (In Press).

Saunders, P. J. W. 1973. Effects of Atmospheric Pollution on Leaf Surface Microflora. Pestic. Sci., 4:589-595.

Wodzinski, R. S., and M. Alexander. 1978. Effects of Sulfur Dioxide on Algae. J. Environ. Qual., 7(3):358-360.

SECTION 6

IMPACT OF SO_2 EXPOSURE ON THE RESPONSE OF WESTERN WHEATGRASS (*AGROPYRON SMITHII*) TO DEFOLIATION

W. K. Lauenroth, J. K. Detling and J. L. Dodd

ABSTRACT

Agropyron smithii populations exposed to three controlled SO_2 concentrations were defoliated either once or twice during the growing season at either a light or heavy rate.

Interactions between SO_2 exposure and defoliation occurred with respect to biomass, number of tillers and sulfur uptake.

INTRODUCTION

During the first four years of a field experiment regarding the response of a North American grassland to controlled low concentrations of sulfur dioxide (Heitschmidt *et al.*, 1978) we have accumulated evidence that exposures during the growing season had subtle but potentially important effects upon the system (Lauenroth and Heasley, 1980). For example, SO_2 exposure reduced chlorophyll concentrations and increased sulfur contents in several important species (Lauenroth *et al.*, 1979; Lauenroth and Dodd, In Prep.), decreased the functional lives of leaves of the dominant species, western wheatgrass (*Agropyron smithii* Rydb.) (Heitschmidt *et al.*, 1978) and decreased the amount of carbon stored in the rhizomes of western wheatgrass (Lauenroth and Heasley, 1980).

Growth initiation of western wheatgrass in the spring and after defoliation is dependent upon carbohydrates stored in rhizomes and roots (Bokhari, 1977). Before the SO_2 treatments were begun the area had been grazed by cattle. Following exclusion of cattle rhizome biomass increased significantly on the Control. In contrast, rhizome biomass failed to recover on the 8.0 pphm SO_2 treatment, suggesting that SO_2 exposure was having an effect similar to grazing. Populations under stress are often more susceptible to damage caused by additional perturbations (Weinstein and McCune, 1979). Because of the importance of these grasslands to the regional livestock economy and the high probability that coal combustion for electric power production will increase in this area we designed a field experiment to examine the potential interactions between defoliation and SO_2 exposure on the native vegetation.

MATERIALS AND METHODS

This experiment utilized a split-split-plot design with SO_2 as the main treatments and defoliation intensity as split-plots and defoliation frequency as the split-split-plots. The three defoliation intensities were (none, light, and heavy) and the two frequencies were (once or twice per season). Each of the SO_2 treatment plots encompassed 0.52 ha and within each were located five replications of each defoliation treatment. Each defoliation treatment was applied to one half square meter.

The heavy defoliation treatment consisted of hand clipping all of the live aboveground biomass and the light treatment removed 50 percent. The single defoliation occurred on 20 May when aboveground live biomass of western wheatgrass is typically near 30 percent of the growing season maximum (Dodd *et al.*, 1979. The second defoliation treatment occurred on 20 June, near the time of expected peak live biomass.

All experimental plots were harvested on 15 August. Aboveground biomass was clipped at the soil surface and separated by species. Each sample was then oven-dried at 60°C for at least 72 hours and weighed. At the time of harvesting the number of tillers of western wheatgrass were counted in each plot. Subsamples of western wheatgrass were analyzed for total sulfur using a Leco Induction Furnace (Laboratory Equipment Co., St. Joseph, MI.).

The data were subjected to a split-split-plot analysis of variance of SO_2 treatments and defoliation intensity and frequency (Table 6.1). Differences between individual means were tested using Tukey's Q procedure (Snedecor and Cochran, 1967).

RESULTS

On 15 August, total aboveground biomass (all species) was significantly ($P < 0.01$) altered by the interaction of clipping intensity and clipping frequency. The main effects and interactions with SO_2 were nonsignificant Total aboveground biomass was unchanged as a result of the single clipping regardless of the intensity (Figure 6 .1). Reapplication of the clipping treatments resulted in significant decreases in total biomass at both clipping intensities.

Biomass of western wheatgrass responded significantly to the interactions of SO_2 × clipping frequency ($P = 0.05$) and clipping intensity × clipping frequency ($P = 0.001$). The latter indicated that standing crop of western wheatgrass was unchanged by the single clipping but significantly decreased by reclipping at both the moderate (50 percent decrease) and heavy (90 percent decrease) intensities (Figure 6.2a). The SO_2 x clipping frequency interaction indicated that western wheatgrass responded differently to SO_2 as a result of being clipped once or twice (Figure 6.2b). The single defoliation resulted in significant decreases in western wheatgrass biomass at the Medium and High SO_2 concentrations compared to the Control. As a result of reapplying the

TABLE 6.1. RESULTS OF ANALYSIS OF VARIANCE

Source of variation	df
SO_2	3
Rep (SO_2)	16
Defoliation Intensity (DI)	2
DI × SO_2	6
Error B	32
Defoliation Frequency (DF)	1
DF × SO_2	3
DF × DI	2
DF × SO_2 × DI	6
Error C	48
Total	119

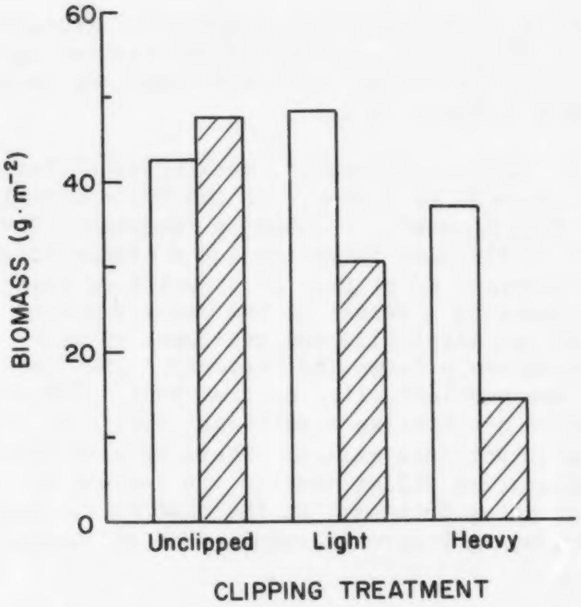

Figure 6.1. Response of total aboveground biomass to three defoliation intensities and two defoliation frequencies (▭ = once, ▨ = twice).

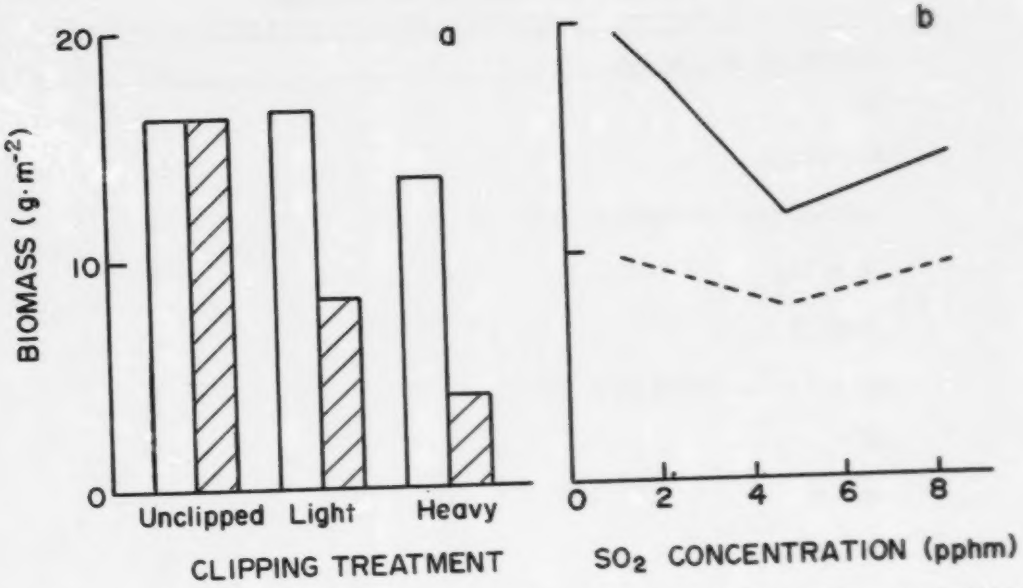

Figure 6.2. Response of western wheatgrass biomass (a) to three defoliation intensities and to two defoliation frequencies (☐ = once, ▨ = twice); (b) to SO_2 concentration at two defoliation frequencies (——— = once, ------ = twice).

clipping treatments on 20 June, the significant differences among SO_2 treatments which were apparent after the single clipping were no longer present. This was largely the result of the large impact of reapplying the heavy clipping treatment (Figure 6.2a).

Analysis of variance of western wheatgrass tiller density identified significant differences as a result of the SO_2 × clipping frenquency ($P = 0.08$), and clipping intensity × clipping frequency ($P = 0.001$) interactions. The interaction of clipping intensity and a single defoliation resulted in a nonsignificant increase in tillers as a result of light clipping and a significant increase as a result of the heavy clipping (Figure 6.3a). Reapplication of the light clipping treatment resulted in no change in tiller density, but there was a large (65 percent) significant decrease following reapplication of the heaviest clipping treatment. The SO_2 x clipping frequency interaction, while not fitting traditional limits of significance ($P \leq 0.05$), does provide important information. These results indicated (Figure 6.3b) that western wheatgrass tiller density was decreased by all SO_2 treatments regardless of clipping intensity at the time of the initial clipping. Reapplication of the clipping treatment resulted in additional decreases in tiller density.

Sulfur concentration in western wheatgrass was significantly altered by the three-way interaction of SO_2, clipping intensity and clipping frequency ($P = 0.02$). Sulfur content was closely related to SO_2 concentration regardless of clipping intensity or frequency (Figure 6.4). A single clipping treatment

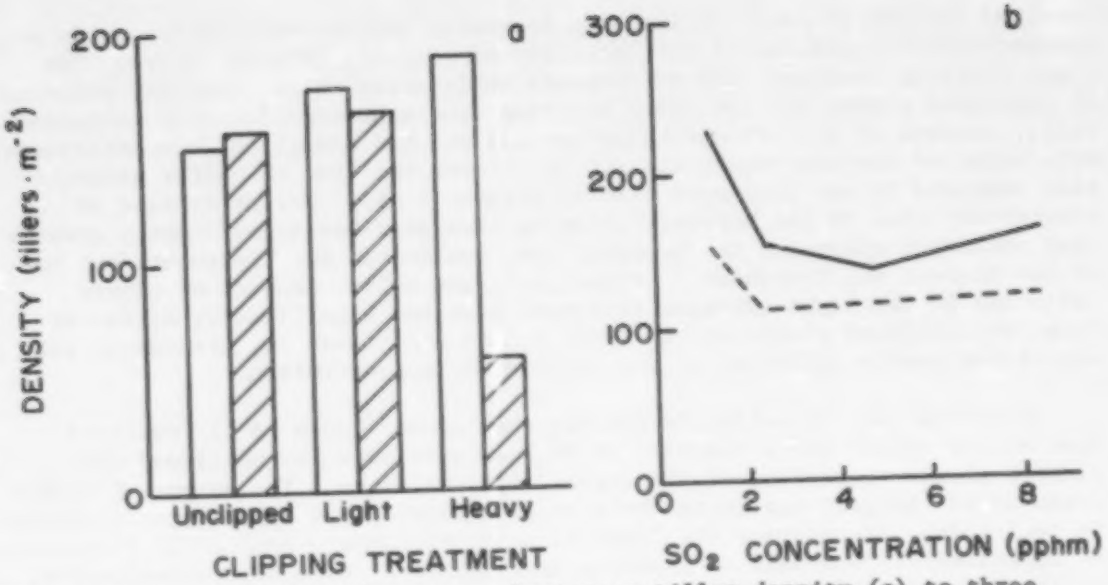

Figure 6.3. Response of western wheatgrass tiller density (a) to three defoliation intensities and two defoliation frequencies (▭ = once, ▨ = twice); (b) to SO_2 concentrations at two defoliation frequencies (——— = once, ------ = twice).

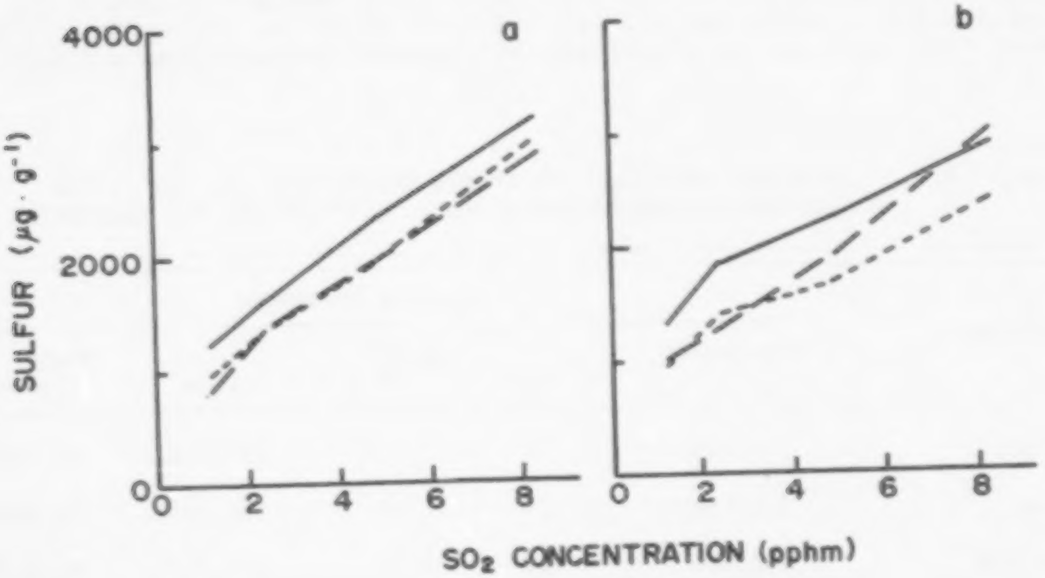

Figure 6.4. Response of sulfur concentration in western wheatgrass to SO_2 concentration, defoliation intensity and one (a) or two (b) defoliations (------ = unclipped, — - — = light, ——— = heavy).

resulted in similar rates of increase in sulfur content with increases in SO_2 concentration regardless of the intensity of clipping (Figure 6.4a). The light clipping treatment did not significantly alter sulfur content, compared to unclipped plants but the heavy clipping treatment significantly increased sulfur content of western wheatgrass at all but the lowest SO_2 concentrations. Reclipping of western wheatgrass plants altered the rate of sulfur accumulation compared to the unclipped plants (Figure 6.4b). Sulfur content of plants subjected to the heaviest clipping treatment was significantly greater than unclipped plants at the Control, Low, and Medium SO_2 treatments but not at the Highest SO_2 treatment. In contrast, the sulfur content of plants subjected to the light clipping treatment were not significantly different from the unclipped plants at the Control, Low and Medium SO_2 treatments but were signficantly different at the Highest SO_2 concentration.

Standing crop of sulfur in western wheatgrass (Table 6.2) indicated that sulfur uptake was a function of SO_2 concentration for unclipped and clipped plants regardless of the degree of defoliation. The amount of sulfur taken up was largely unaffected by a single clipping and substantially altered by reclipping. Reapplying the light clipping treatment resulted in a decrease of approximately one-half in standing crop sulfur at each SO_2 concentration. At the heaviest clipping intensity reclipping decreased the standing crop of sulfur on the Control and Low SO_2 treatments by a factor of 8 and on the Medium and High SO_2 treatments by a factor of 6.

The rate of sulfur uptake (standing crop divided by accumulation period) could not be calculated for the light defoliation treatment because of the complications posed by the stubble. Uptake of sulfur was doubled by a single heavy defoliation at the Control and Low treatment concentrations and increased

TABLE 6.2. STANDING CROP (mg) AND SULFUR UPTAKE RATE (mg day^{-1}) FOR WESTERN WHEATGRASS SUBJECTED TO CLIPPING AND SO_2 TREATMENTS

SO_2 Treatment	Clipping Treatment				
	Unclipped*	Light		Heavy	
		Once	Twice	Once	Twice
Control	17(.125)	18	9	19(.218)	2(.036)
Low	21(.154)	26	13	25(.287)	3(.054)
Medium	24(.176)	23	11	25(.287)	4(.072)
High	44(.324)	48	22	36(.414)	6(.107)

* All unclipped results combined.

by 50 percent at the Medium and High SO_2 concentrations. Reapplying the heavy defoliation sharply decreased rate of uptake to one-third of that calculated for unclipped plants.

DISCUSSION

While we observed important interactions between SO_2 exposure and defoliation treatments, the majority of our results indicated that the intensity and frequency of defoliation were most influencial in determining the growth and tillering responses we observed. Interactions between SO_2 and either biomass (Figure 6.2b) or tiller density (Figure 6.3b) of western wheatgrass represent potentially important indications of the state of the system after exposure to SO_2. Whether these results would be compensated for or compounded by subsequent seasons of SO_2 exposure and defoliation must be determined before the long-term consequences of these interactions can be determined. In this regard, it is interesting to note that a single clipping applied early in the growing season had no significant effect on final total biomass of all species (Figure 6.1) or that of western wheatgrass (Figure 6.2a). Although direct estimates of aboveground net primary production (ANPP) were not made, these results suggest that ANPP was stimulated by a single defoliation early in the season, regardless of intensity. Since estimates of amount of shoot material removed at each clipping were not made, it is not possible to infer confidently the effects of two defoliations on ANPP. In spite of many reports of declines in ANPP following grazing (Jameson, 1963), reports of compensatory growth, or even increases in plant yield following light to moderate levels of defoliation, are common (McNaughton, 1979; Harris, 1974). Such compensatory growth following grazing in grasslands may result from a number of indirect effects on microclimate, such as increasing light penetration to lower leaves in the canopy or reducing evapotranspiration and prolonging the period of favorable soil moisture during drought (McNaughton, 1979). In addition, individual plants frequently respond to defoliation by increasing photosynthetic rates in remaining undamaged leaves or newly developing leaves (Detling *et al.*, 1979; Painter and Detling, 1980), and increasing the proportion of current photosynthate allocated to synthesis of new leaves (Detling *et al.*, 1980 Ryle and Powell, 1975). Under laboratory conditions, western wheatgrass did not appear to change its photosynthate allocation patterns in response to differential tiller defoliation, however, (Painter and Detling, 1980).

The tillering response of grasses to defoliation is probably affected by interactions among internal factors, such as stage of development and hormone concentration, and external environmental factors, such as light, temperature, or photoperiod (Goodin, 1972; Laude, 1972). Thus, when defoliation results in removal of only leaves, tiller production is often depressed since available carbohydrates are apparently utilized for production of new tillers only if the demand for the growth of current leaves has been met already (Youngner, 1972). Tillering may be enhanced by defoliation, however, if apical meristems are removed, and hence apical dominance is destroyed (Youngner, 1972). Under laboratory conditions, western wheatgrass produced about the same number of tillers regardless of level of defoliation up to removal of 75 percent of the tillers (Painter and Detling, 1980), a finding which is generally consistent with the relatively small change in tiller density under all clipping treatments except in those plots receiving two complete defoliations (Figure 6.4).

Defoliation intensity and frequency produced a clear interaction with SO_2 exposure in influencing sulfur uptake (Figure 6.4). We would expect this to be of short-term importance only if increased sulfur content of the forage influenced forage palatability or digestability. If either are affected, exposure to SO_2 will be most important in determining this response. The small increment in sulfur concentration as a result of clipping will be of lesser importance. McNary (1980) found that grasshoppers discriminate against western wheatgrass plants grown on the SO_2 treatments. The explanation for this may reside in their differing sulfur contents. Rumsey (1978) reported reduced intake by cattle of a feed high in sulfur.

At the beginning of the experiment we hypothesized that the lack of recovery of rhizome biomass, after protection from grazing, was an indication that SO_2 exposure was creating a condition of stress within the western wheatgrass population (Esch *et al.*, 1975; Lauenroth *et al.*, 1978). Additionally we believed that by subjecting the population to an additional stress or (defoliation) we would observe a large negative response which would be proportional to the amount of stress resulting from SO_2 exposure. To a large degree the response we observed did not support these hypotheses. Perhaps a single season of defoliation was not sufficient to produce the expected response. It is also possible that rhizome biomass is not as sensitive an indicator as we believed. As a result of a single season investigation of the interactions between SO_2 exposure and defoliation it appears that increased sulfur loading of system components may be the most significant response threatening long-term system stability.

CONCLUSIONS

Subjecting an *Agropyron smithii* population, which was hypothesized to be in a stressed condition as a result of SO_2 exposure, to the additional perturbation of defoliation produced only a few responses which would not have been expected from defoliation alone.

Increased sulfur loading of system components may be the most important response in terms of long-term system integrity.

REFERENCES

Bokhari, U. G. 1977. Regrowth of Western Wheatgrass Utilizing ^{14}C Labeled Assimilates Stored in Belowground Parts. Plant and Soil, 48:115-127.

Detling, J. K., M. I. Dyer, and D. T. Winn. 1979. Net Photosynthesis, Root Respiration, and Regrowth of *Bouteloua gracilis* Following Simulated Grazing. Oecologia, 41:127-134.

Detling, J. K., M. T. Dyer, C. Procter-Gregg, and M. T. Winn. 1980. Plant-herbivore Interactions: Examination of Potential Effects of Bison Saliva on Regrowth of *Bouteloua gracilis* (H.B.K.) Lag. Oecologia.

Dodd, J. L., W. K. Lauenroth, G. L. Thor, and M. B. Coughenour. 1978. Effects of Chronic Low Level SO_2 Exposure on Producers and Litter Dynamics. In: E. M. Preston and T. L. Gullett (eds.) The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana. EPA-600/3-79-044. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 384-393.

Esch, G. W., J. W. Gibbons and J. E. Bourque. 1975. An Analysis of the Relationship Between Stress and Parasitism. Amer. Midl. Nat. 93:339-353.

Goodin, J. K. 1972. Chemical Regulation of Growth in Leaves and Tillers. pp. 135-145. In: V. B. Youngner and C. M. McKell (eds.) The Biology and Utilization of Grasses, pp. 292-303. New York and London, Academic Press.

Harris, P. 1974. A Possible Explanation of Plant Yield Increases Following Insect Damage. Agro-ecosystems, 1:219-225.

Heitschmidt, R. K., W. K. Lauenroth, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulfur Dioxide on Western Wheatgrass on a Southeastern Montana Grassland. J. Appl. Ecol., 14:859-868.

Jameson, D. A. 1963. Responses of Individual Plants to Harvesting. Bot. Rev., 29:532-594.

Laude, H. M. 1972. External Factors Affecting Tiller Development. pp. 164-154. In: V. B. Youngner and C. M. McKell, (eds.) The Biology and Utilization of Grasses, pp. 292-303. New York and London, Academic Press.

Lauenroth, W. K., J. L. Dodd and P. L. Sims. 1978. The Effects of Water and Nitrogen Induced Stresses on Plant Community Structure in a Semi-arid Grassland. Oecologia, 36:211-222.

Lauenroth, W. K., and J. E. Heasley. 1980. Impact of Sulfur Deposition on Grassland Ecosystem. In: D. S. Shriner (ed.) Atmospheric Sulfur Deposition: Environmental Impact and Health Effects. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Lauenroth, W. K., C. J. Bicak, and J. L. Dodd. 1979. Sulfur Accumulation in Western Wheatgrass Exposed to Three Controlled SO_2 Concentrations. Plant and Soil, 53:131-136.

Lauenroth, W. K., and J. L. Dodd. In Prep. Chlorophyll Reduction in Western Wheatgrass Exposed to Sulfur Dioxide.

McNary T. J., J. W. Leetham, W. K. Lauenroth, and J. L. Dodd. 1980. Response of Rangeland Grasshopper Populations to Controlled Levels of SO_2. In: E. M. Preston and D. W. O'Guinn (eds.) The Bioenvironmental Impact of a Coal-fired Power Plant, Fifth Interim Report, Colstrip, Montana. EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 272-278.

McNaughton, S. J. 1979. Grazing as an Optimization Process: Grass-ungulate Relationships in the Serengeti. Am. Nat., 113:691-703.

Painter, E. L..and J. K. Detling. 1980. Effects of Defoliation on Net Photosynthesis and Regrowth of Western Wheatgrass. J. Range Manag.

Rumsey, T. S. 1978. Effects of Dietary Sulfur Addition and Synovex-S in Ear Implants on Feedlot Steers Fed an All-concentrate Finishing Diet. J. An. Sci., 46(2):463-477.

Ryle, G. J. H. and C. E. Powell. 1975. Defoliation and Regrowth in the Graminaceous Plant: The Role of Current Assimilate. Ann. Bot., 39:297-310.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State Univ. Press, Ames.

Weinstein, L. H., and D. C. McCune. 1979. Air Pollution Stress. In: Crop Plants. H. Mussell and R. Staples (eds.) John Wiley and Sons, Inc.

Youngner, V. B. 1972. Physiology of Defoliation and Regrowth. In: The Biology and Utilization of Grasses. V. B. Youngner and C. M. McKell (eds.) New York and London, Academic Press. pp. 292-303.

SECTION 7

RESPONSE OF *BOUTELOUA GRACILIS* TO CONTROLLED SO_2 EXPOSURE

W.K. Lauenroth, J.K. Detling, C.J. Bicak, and J.L. Dodd

ABSTRACT

Bouteloua gracilis, an important warm season grass native to the Great Plains of North America, was grown hydroponically and exposed under field conditions to controlled concentrations of SO_2. After 31 days of exposure individual plants were harvested and biomass accumulation and sulfur content were measured.

We found no impact of SO_2 on live shoot weights, root weights, shoot:root ratios or number of tillers. Significant differences were found for crown weights and the ratios of live to dead shoot weights.

INTRODUCTION

Early investigations regarding the effects of sulfur dioxide on plant growth focused largely upon short-term (hours) high concentration impacts (Thomas, 1961). Recently, a number of workers have investigated physiological impacts of low concentrations for long time periods (weeks to months) (Ashenden and Mansfield, 1977; Tingey and Reinert, 1975; Crittenden and Read, 1979; Bell *et al.*, 1979). Sulfur dioxide impacts on grasslands are receiving considerable attention in Great Britain largely because of the importance of a single SO_2 sensitive species, *Lolium perenne* L. Although there is not general agreement about its specific responses or the threshold concentrations which elicit those responses, there is general agreement that perennial ryegrass responds to low concentrations of SO_2 (Cowling *et al.*, 1973; Bell and Clough, 1973; Bell *et al.*, 1979).

North American grasslands are considerably more diverse than those in Great Britain (Lauenroth, 1979) and have only recently received attention with regard to potential impacts of SO_2 (Heitschmidt *et al.*, 1978; Ferenbaugh, 1978; Coughenour *et al.*, 1979). Coincidentally, all of this work has been focused upon the responses of C_3 grasses to sulfur dioxide. Because stomatal behavior is very important in determining responses of plants to SO_2 exposure (Winner and Mooney, 1980) the differences in stomatal control of photosynthesis observed between C_3 and C_4 plants (Körner *et al.*, 1979) may result in quite different responses to SO_2. Our objectives here are to report an experiment which was designed to utilize a unique field exposure facility (Heitschmidt *et al.*, 1978) to evaluate the impact of SO_2 on the growth of a native C_4 grass *Bouteloua gracilis* H.B.K. Lag. *Bouteloua gracilis* is an important dominant over much of the Central and Southern Great Plains of North America and is an important co-dominant on many sites in the Northern Great Plains (Lauenroth *et al.*, 1980).

MATERIALS AND METHODS

Bouteloua gracilis plants were grown from seeds in flats containing vermiculite until they were 30-days old. At that time they were sorted for a uniform size and placed in 12-liter hydroponic containers with 0.143-strength Hoaglands solution (Detling *et al.*, 1979). Sponge-stoppers held the 12 plants upright in each container. The experiment was begun on 13 July when one container was placed in each of the SO_2 treatment plots and progressed until 15 August. Containers with hydroponically grown *Bouteloua gracilis* plants were randomly located within each SO_2 treatment and buried so that plants had a normal position within the grassland canopy. Nutrient solutions were replenished daily and the entire contents of the containers were changed each week. It was necessary to cover the containers with a wire mesh to prevent grazing by grasshoppers.

At the end of the experiment tillers were counted and plants were oven-dried at 60°C until they reached a constant weight. Each plant was then separated into four components, 1) live shoots, 2) dead shoots, 3) crowns, and 4) roots. Dry weights were measured for each component. Sub-samples of live aboveground and live belowground material were analyzed for total sulfur content using a Leco Induction Furnace (Laboratory Equipment Co.).

The results were statistically analyzed using analysis of variance. Tukey's Q values were used to calculate least significant ranges (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSSION

After 31 days exposure of *Bouteloua gracilis* to controlled levels of sulfur dioxide in the field, we found no statistically significant ($P = 0.05$) changes in live shoot weights, root weights, shoot:root ratios or number of tillers (Table 7.1). Significant differences were found for crown weights (Table 7. 1) ($P \leq 0.03$) and the ratio of live to dead shoot weights (Figure 7.1) ($P \leq 0.001$).

Bouteloua gracilis is a hemicryptophyte with the crowns representing the origin of the perennating buds as well as an important storage location for labile carbohydrates. The potential long-term implications of reduced crown biomass include a reduction in the number of active stem meristems and a decreased supply of carbohydrates for growth initiation and regrowth after defoliation. Lauenroth and Heasley (1979) reported a similar effect in the C_3 cryptophyte *Agropyron smithii* Rybd. Exposure to SO_2 reduced the amount of carbon stored in rhizomes. Tingey and Reinert (1975) found a much larger impact of SO_2 exposure on *Medicago sativa* L. roots than shoots. Although a

TABLE 7.1. DRY WEIGHTS OF LIVE SHOOTS, ROOTS AND CROWNS, SHOOT:ROOT RATIOS AND NUMBER OF TILLERS PER PLANT FOR *BOUTELOUA GRACILIS* EXPOSED TO FOUR CONTROLLED LEVELS OF SO_2

	Plant Part				
SO_2 Conc. (pphm)	Shoot (mg)	Root (mg)	Crown (mg)	Shoot:Root	Number of tillers
<0.85	588	284	290	1:99	40
2.1	657	247	293	2:66	34
4.4	605	244	223	2:45	31
6.5	514	252	193	2:79	35
S.E.(11 df)	75	30	29	0:44	3

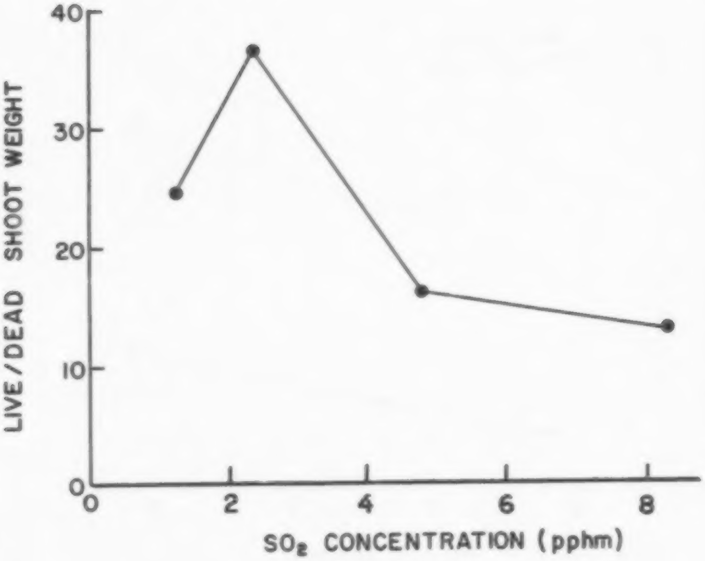

Figure 7.1. Ratio of live to dead shoot weights of *Bouteloua gracilis* exposed to SO_2 treatments.

definitive translocation study showing reduced carbon transport to perennating and/or belowground organs has not been reported, circumstantial evidence supports this hypothesis.

At the lowest concentration of SO_2 the live to dead shoot biomass ratio was increased significantly ($P = 0.05$) while at the two highest concentrations a significant reduction in the ratio was observed. Heitschmidt *et al.* (1978) reported an increase in the number of live leaves per plant of *Agropyron smithii*, as a result of exposure to SO_2 and a decrease in the proportion of dead leaves in July for the 2.1 pphm treatment. In addition they found increased proportions of dead leaves at the 4.4 and 6.5 pphm treatments in June. Ferenbaugh (1978) found that the C_3 grass, *Oryzopsis hymenoides* Roem. & Schult. was unaffected by SO_2 concentrations below 13.5 pphm except for a decrease in total chlorophyll content at 6.2 pphm. Cowling and Koziol (1978) exposed another C_3 grass, *Lolium perenne*, to three controlled SO_2 concentrations, beginning on the 41st day after germination and continuing for 49 days. They found no significant effects on dry weight of shoots, specific leaf area, net photosynthesis, dark respiration or transpiration coefficient. Following harvesting and 21 days of regrowth they found only a small significant decrease in the specific leaf area at the 15.4 pphm treatment. These findings contrasted those of an early study with the same species and the same concentrations of SO_2 in which the yield of shoots was reduced (Lockyer *et al.*, 1976).

Sulfur concentration in shoots of *Bouteloua gracilis* not exposed to the SO_2 treatments was 1800 $\mu g \cdot g^{-1}$ (Figure 7.2). Exposure to the high concentration treatment significantly ($P < 0.01$) increased this to 2200 $\mu g \cdot g^{-1}$.

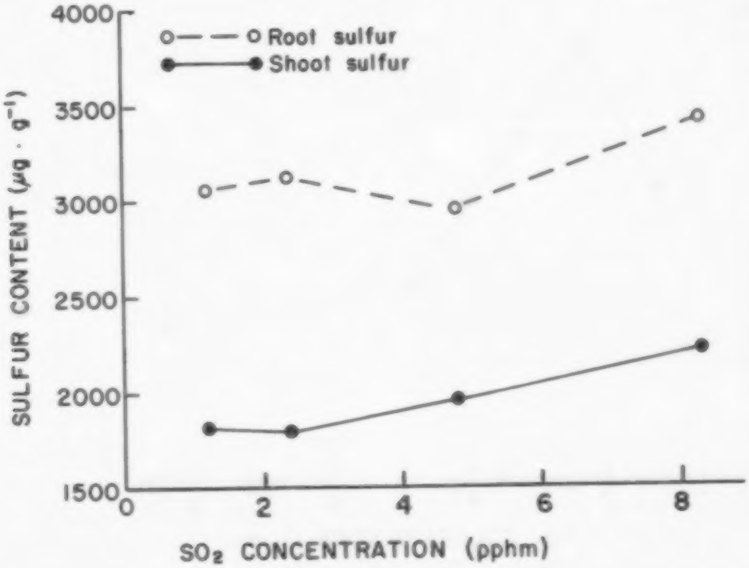

Figure 7.2. Shoot and root sulfur content of *Bouteloua gracilis* exposed to three controlled SO_2 treatments.

Lauenroth *et al.* (1979) reported that average shoot sulfur contents of *Agropyron smithii*, indigenous to the experimental site, ranged from 800 to 1100 $\mu g \cdot g^{-1}$. Exposure to the High SO_2 treatment increased these values at the rate of 385-650 $\mu g \cdot g^{-1}$ $month^{-1}$. End of the growing season sulfur concentrations were in the range of 3000-5000 $\mu g \cdot g^{-1}$. Root sulfur concentrations for blue grama were higher than shoot concentrations by approximately 60 percent (Figure 7.2). Bicak *et al.* (In Prep.) reported root and shoot sulfur concentrations for *Agropyron smithii* grown in a similar hydroponic system and exposed to SO_2 concentrations in the control and high treatments. Shoot sulfur content was approximately 20 percent greater than root sulfur content after a 6-day exposure period in June. Shoot and root sulfur contents were equal after a 6-day exposure in July. Cowling and Lockyer (1978) reported no effect of SO_2 exposure on the root sulfur content of *Lolium perenne* and shoot sulfur contents were 2 to 3 times larger than roots.

CONCLUSIONS

Our hypothesis that differences in physiological behavior between C_4 and C_3 plants would result in different responses to SO_2 exposure was not substantiated by results from the experiment. Responses of C_4 grass *Bouteloua gracilis* were largely similar to those previously reported for C_3 grasses.

REFERENCES

Ashenden, T. W., and T. A. Mansfield. 1977. Influence of Wind Speed on the Sensitivity of Ryegrass to SO_2. J. of Exp. Bot., 28:729-735.

Bell, J. N. B., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulfur Dioxide. Nature, (London) 241:47-49.

Bell, J. N. B., A. J. Rutter, and J. Relton. 1979. Studies on the Effects of Low Levels of Sulfur Dioxide on the Growth of *Lolium perenne* L. New Phytol., 83:627-643.

Bicak, C. J., W. K. Lauenroth, J. L. Dodd. In Prep. Sulfur Distribution and Allocation in Western Wheatgrass Exposed to SO_2 Under Variable Nutrient Sulfur Regimes. In: Bioenvironmental Impact of a Coal-fired Power Plant. Sixth Interim Report, Colstrip, Montana. E. M. Preston, D. W. O'Guinn, and R. A. Wilson (eds.) U.S. Environmental Protection Agency, Corvallis Oregon.

Coughenour, M. B., J. L. Dodd, D. C. Coleman, and W. K. Lauenroth. 1979. Partitioning of Carbon and SO_2 in a Native Grassland. Oecologia, 42: 229-240.

Cowling, D. W., L. H. P. Jones, and D. R. Lockyer. 1973. Increased Yield Through Correction of Sulfur Deficiency in Ryegrass Exposed to Sulfur Dioxide. Nature, (London) 243:479-480.

Cowling, D. W., and M. J. Koziol. 1978. Growth of Ryegrass (*Lolium perenne* L.) Exposed to SO_2. I. Effects on Photosynthesis and Respiration. J. of Exp. Bot., 29:1029-1036.

Cowling, D. W., and D. R. Lockyer. 1978. The Effect of SO_2 on *Lolium perenne* L. Grown at Different Levels of Sulfur and Nitrogen Nutrition. J. Exp. Bot., 29:257-265.

Crittenden, P. D., and D. J. Read. 1979. The Effects of Air Pollution on Plant Growth with Special Reference to Sulfur Dioxide. III. Growth Studies with *Lolium multiflorum* lam. and *Dactylis glomerata* L. New Phytol., 83:645-651.

Detling, J. K., M. I. Dyer, and D. T. Winn. 1979. Net Photosynthesis, Root Respiration, and Regrowth of *Bouteloua gracilis* Following Simulated Grazing. Oecologia, 41:127-134.

Ferenbaugh, R. W. 1978. Effects of Prolonged Exposure of *Oryzopsis hymenoides* to SO_2. Water, Air, and Soil Pollut., 10:27-31.

Heitschmidt, R. K., W. K. Lauenroth, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulfur Dioxide on Western Wheatgrass in a Southeastern Montana Grassland. J. of Appl. Ecol., 14:859-868.

Körner, C., J. A. Scheel and H. Bauer. 1979. Maximum Leaf Diffusive Conductance in Air Plants. Photosynthetica, 13:45-82.

Lauenroth, W. K. 1979. Grassland Primary Production: North American Grasslands in Perspective. In: N. R. French (ed.) Perspectives in Grassland Ecology. Ecological Studies. Vol. 32, Springer Verlag, New York. pp. 3-24.

Lauenroth, W. K., C. J. Bicak, and J. L. Dodd. 1979. Sulfur Accumulation in Western Wheatgrass Exposed to Three Controlled SO_2 Concentrations. Plant and Soil, 53:131-136.

Lauenroth, W. K., J. L. Dodd, and C. E. Dickinson. 1980. Aboveground Biomass Dynamics of Blue Grama in a Short-grass Steppe and an Evaluation of a Method for Separating Live and Dead. J. Range Manage. (In press).

Lauenroth, W. K., and J. E. Heasley. 1979. Impact of Sulfur Deposition on Grassland Ecosystems. Symposium on Potential Environmental and Health Effects of Atmospheric Sulfur Deposition. Oak Ridge National Laboratory, Life Sciences Symposium Series. 14-18 October, 1979. Gatlinburg, Tennessee.

Lockyer, D. R., D. W. Cowling, and L. H. P. Jones. 1976. A System for Exposing Plants to Atmosphere Containing Low Concentrations of Sulfur Dioxide. J. Exp. Bot., 27:397-409.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco. 776 p.

Thomas, M. D. 1961. Effects of Air Pollution on Plants. In: Air Pollution. Monograph Ser. W. H. O., No. 46 Geneva. pp. 233-278.

Tingey, D. D., and R. A. Reinert. 1975. The Effect of Ozone and Sulfur Dioxide Singly and in Combination on Plant Growth. Environ. Pollut., 9: 117-125.

Winner, W. E., and H. A. Mooney. 1980. Ecology of SO_2 Resistance. I. Effects of Fumigations of Gas Exchange of Deciduous and Evergreen Shrubs. Oecologia. (In Press).

SECTION 8

THE IMPACT OF SULFUR DIOXIDE ON THE CHLOROPHYLL CONTENT OF GRASSLAND PLANTS

W. K. Lauenroth and J. L. Dodd

ABSTRACT

Chlorophyll *a* and *b* contents were measured by extraction in ethanol for eight species native to northern Great Plains grasslands in North America. Chlorophyll *a* was most sensitive to SO_2 exposure but the degree of sensitivity was species specific. Concentrations of both chlorophylls were increased, unchanged or decreased depending upon SO_2 concentration and species. Chlorophyll is not a reliable indicator of SO_2 exposure in grasslands.

INTRODUCTION

Chlorophyll concentrations in plants and plant communities have been shown to be positively related to net photosynthesis and net primary productivity (Patterson *et al.*, 1977; Buttery and Buzzell, 1977; Brougham, 1960; Bray, 1960). In contrast to this Ovington and Lawrence (1967) and Sanger (1971) disputed the contention that chlorophyll concentrations and net primary productivity were closely coupled explaining that chlorophyll expressed on either a leaf area or dry weight basis varied widely throughout the growing season. Ovington and Lawrence (1967) reported dry-weight ratios of chlorophyll to organic matter for a maize field ranged from a maximum of 19 early in the growing season to nine late in the growing season. Sanger (1971) suggested that before any meaningful relationships between productivity and chlorophyll content could be made one should be aware of the time of year and the duration and magnitude of fluctuation of maximum chlorophyll content of the major components of the plant community. Although some studies have found simple relationships between chlorophyll content and either net photosynthesis or net primary productivity and others have not found a simple relationship, none deny the physiological connection between chlorophyll concentration and the potential of a plant or plant community to carry on photosynthesis.

Chlorophyll concentration has been found to be related to many aspects of a plant's natural environment (Sanger, 1971) as well as too many by-products of man's activities (Knudson *et al.*, 1977; Beckerson and Hofstraw, 1979; Rabe and Kreeb, 1979). Sulfur dioxide (SO_2) is an important constituent of anthropogenic air pollution which has been shown to cause decreases in chlorophyll concentrations over a wide range of plant species (Malhotra, 1977; Lauenroth and Dodd, submitted). Beckerson and Hofstraw (1979) exposed white bean plants to concentrations of 15 pphm SO_2 for 5 days and found significant increases in both chlorophyll *a* and chlorophyll *b* after 2 days exposure to SO_2. Chlorophyll *a* content remained higher than the Control throughout the 5 day period and chlorophyll *b* content was significantly reduced by the end of the 5 day period. They offered no explanation for this phenomena. Rabe and Kreeb (1979) exposed seven different plant species to 5 pphm SO_2 for approximately 1 month. They observed non-significant decreases in chlorophyll content for spring grown alfalfa and for tobacco. Significant decreases were observed for winter barley, tulip, horsebean, turnip and alfalfa grown in the fall. Sulfur dioxide exposure resulted in a non-significant increase in chlorophyll concentration in bush tomatoes. These responses demonstrate that all plants cannot be expected to respond in the same manner to the exposure of SO_2

This investigation was undertaken to determine the responses of a variety of native grassland plants to exposure to SO_2 under field conditions. Previous studies have shown that chlorophyll content of the dominant species in the grassland, *Agropyron smithii* Rydb., was sensitive to exposure to SO_2 (Lauenroth and Dodd, submitted).

MATERIALS AND METHODS

The chlorophyll (chl) content of actively growing stems and leaves of eight species of grasslands plants was assessed using a modification of the methods of Knudson *et al.*, 1977 (Table 8.1). The major modification was substitution of a short homogenizing step in blender instead of the second extraction. After extraction the chopped plant material was collected on filter paper, oven-dried and weighed. Chlorophyll is expressed on a dry weight basis. Sample dates were chosen to coincide with periods of maximum vegetative growth for each species. Twenty-five samples of each species were collected from each SO_2 treatment. Each sample was immediately placed in a 30- ml vial of ethanol, kept in the dark and returned to the laboratory for analysis within 24 hours (Lauenroth and Dodd, submitted).

Plant species selected for chl determination represented those which were most abundant in the plant community and those with specialized functional roles. *Agropyron smithii* is the dominant species in the grassland composing 40 and 60 percent of total net primary production. Each sample of this species consisted of three leaves. *Koeleria cristata* is the second most abundant grass in the community and each sample consisted of three leaves. *Bromus japonicus* Thunb. is the most important annual grass in the plant community and each sample consisted of six tillers each approximately 5 cm in height. *Taraxacum officinale* is a very early growing perennial forb and each sample consisted of three fully expanded center leaves each approximately 7 cm in length. *Achillea millefolium* is another important forb in the plant community with a slightly later growing period than *T. officinale*. Each sample of this species consisted of one plant with approximately three leaves. *Sphaeralcea coccinea* (Pursh) Rybd. is the most important forb in the community

TABLE 8.1. CHLOROPHYLL CONCENTRATIONS* AND *a*:*b* RATIOS UNDER CONTROL CONDITIONS AND PROBABILITIES FROM ANALYSES OF VARIANCE OF RESPONSES OF CHLOROPHYLL *a* AND *b*. LEAST SIGNIFICANT RANGES ARE IN PARENTHESES

					Statistical Results	
Species	Date Sample	Chl *a*	Chl *b*	Chl *a*:*b* (control)	Chl *a*	Chl *b*
Psar	20 June	4.9	1.6	3.0	$P<0.001$ (0.47)	$P<0.001$ (0.25)
Spco	20 June	3.9	1.5	2.6	$P<0.001$ (0.65)	$P=0.64$ (-)
Trdu	20 May	3.0	1.2	2.4	$P=0.09$ (-)	$P=0.02$ (0.19)
Kocr	20 May	5.8	2.3	2.5	$P=0.08$ (-)	$P=0.14$ (-)
Taof	20 May	6.7	3.2	2.1	$P<0.001$ (1.90)	$P<0.001$ (0.93)
Acmi	20 May	2.7	1.3	2.1	$P<0.001$ (0.22)	$P=0.03$ (0.34)
Brja	20 May	15.1	13.2	1.1	$P<0.001$ (4.02)	$P<0.001$ (3.83)
Agsm	20 May	4.6	4.6	1.0	$P<0.001$ (1.27)	$P=0.004$ (1.71)

* ($mg \cdot g^{-1}$)

from the point of view of consumers as it is an important constituent of diets of both antelope and cattle. Each sample of this species was collected from a non-flowering plant and consisted of the center three leaves. *Psoralea argophylla* Pursh is the most important legume in the community and each sample consisted of two leaves with five leaflets each. *Tragapogon dubius* is a very common biennial forb and each sample consisted of one second year plant.

Statistical analysis of the data was by analysis of variance. Tukey's *Q* procedure was used to identify significant differences among treatment means (Sokol and Rohlf, 1969).

RESULTS AND DISCUSSION

The species selected for this experiment represented a wide range in concentrations of chl *a* and *b* (Table 8.1). Chlorophyll *a* concentrations ranged from 2.7 mg g^{-1} for *Achillea millefolium* to 15.1 mg g^{-1} for *Bromus japonicus*. Chlorophyll *b* ranged from 1.2 mg g^{-1} for *Tragapogon dubius* to 13.3 mg g^{-1} for *Bromus japonicus*. Ratios of chl *a* to *b* ranged from a low of 1 for *Agropyron smithii* to a high of 3 for *Psoralea argophylla*. Sanger (1971) reported chl *a*:*b* ratios for three species of deciduous trees ranging from 1.0 to 3.5. Sestak (1971) reviewed the literature regarding measurement of chl *a* and *b* and reported that *a*:*b* ratios are generally 1.5 to 3.5. In addition he found that chls comprise 0.5 to 2.0 percent of the dry weight of plants. Our results ranged from 0.6 to 2.8 percent.

Exposure to SO_2 resulted in significant (Table 8 .1) alterations in chl *a* concentrations in six of the species examined (Figure 8 .1) and significant changes in chl *b* also for six species (Figure 8 .2). Chlorophyll concentration is often cited as a sensitive and reliable indicator of atmospheric sulfur dioxide (Heck *et al.*, 1979; Knabe, 1976; Linzon, 1978). Our results for chls *a* and *b* showed that a wide variety of responses may be expected. Chlorophyll concentration may be a sensitive and reliable indicator of SO_2 exposure in grasslands only if one carefully selects species.

Chlorophyll contents differed among species in quantity, variability, and response to SO_2. *Koeleria cristata* was apparently the most resistant to SO_2 with both chls remaining unchanged regardless of concentration. Careful examination of the data indicates that chl *a* was decreased substantially and chl *b* was decreased a small amount. The lack of statistically significant differences as a result of SO_2 exposure was probably the result of large variability in chl content among leaves and plants. *Agropyron smithii*, *Sphaeralcea coccinea*, and *Achillea millefolium* were affected with regard to chl *a* at one of the SO_2 concentrations, but chl *b* remained unchanged. Chlorophyll *a* has been reported to be more sensitive to SO_2 than chlorophyll *b* (Malhotra, 1977, Peiser and Yang, 1977) although the basis for this difference is not well documented.

The most sensitive species we sampled was *Bromus japonicus* with both chl *a* and *b* significantly reduced by the presence of SO_2. Both chls were reduced equally by the Low and High SO_2 treatments. Additional indications of sensitivity to SO_2 with respect to net production and sulfur accumulation make this species an excellent candidate as a biological indicator of SO_2 air pollution.

The responses of three species, *Psoralea argophylla*, *Tragapogon dubius*, and *Taraxacum officinale*, were peculiar and difficult to interpret. Chlorophyll *a* in all three species was increased by the Low SO_2 treatment, unchanged by the Medium and increased by the High treatment. None of these changes were significant for *T. dubius* and all were significant ($P = 0.05$) for the remaining two species. Chlorophyll *b* had the same pattern. All changes in chl *b* were significant ($P = 0.05$) except the increase on the High treatment for *T. dubius*. Since our experiment was designed to document changes in chl concentrations as a result of SO_2 exposure, we have no explanatory information for this response.

CONCLUSIONS

Chlorophyll concentrations in grassland plants native to northern Great Plains grasslands are not uniformly sensitive to SO_2 exposure. Responses observed for eight species ranged from significant increases to significant decreases. The concept of chl as a sensitive and reliable indicator of SO_2 exposure does not hold for all species in grasslands.

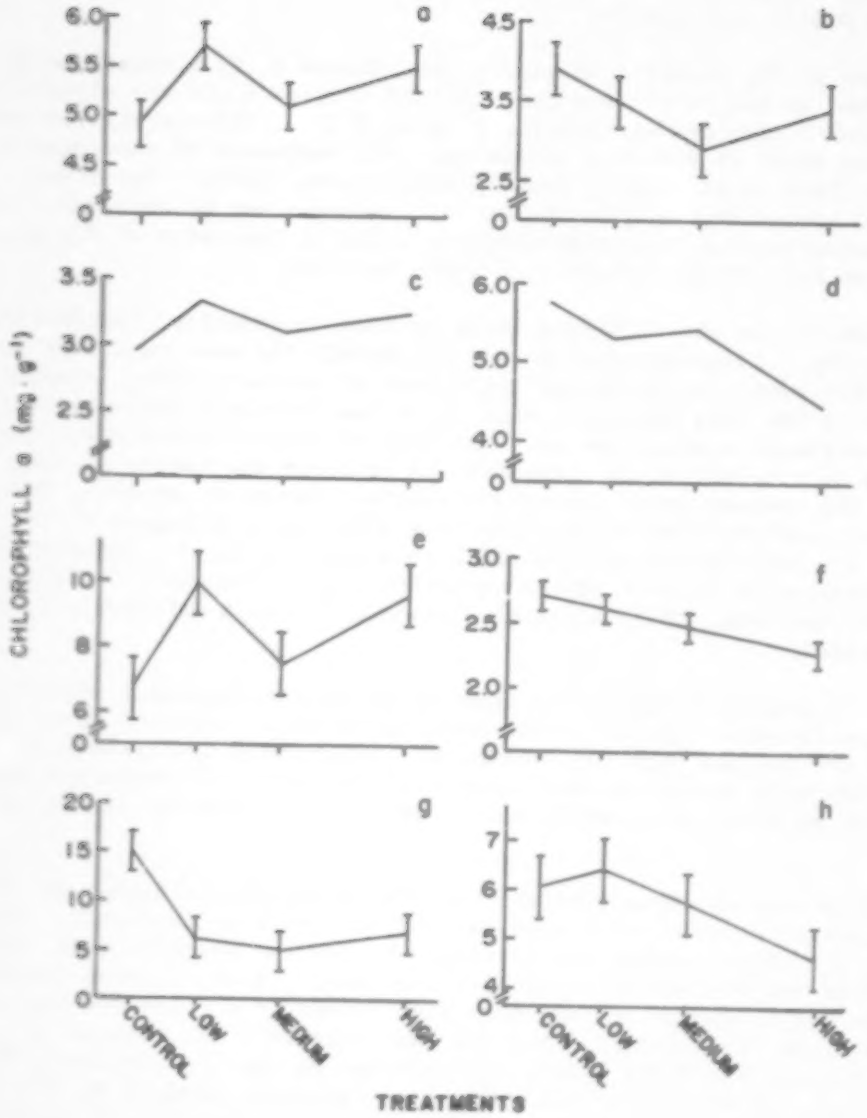

Figure 8.1. Responses of chlorophyll *a* to three concentrations of SO_2 for; a) *Psoralea argophylla*, b) *Sphaeralcea coccinea*, c) *Tragapogon dubius*, d) *Koeleria cristata*, e) *Taraxacum officinale*, f) *Achillea millefolium*, g) *Bromus japonicus*, and h) *Agropyron smithii*. Vertical bars on curves represent least significant ranges (P = 0.05). No bars indicate no significant differences.

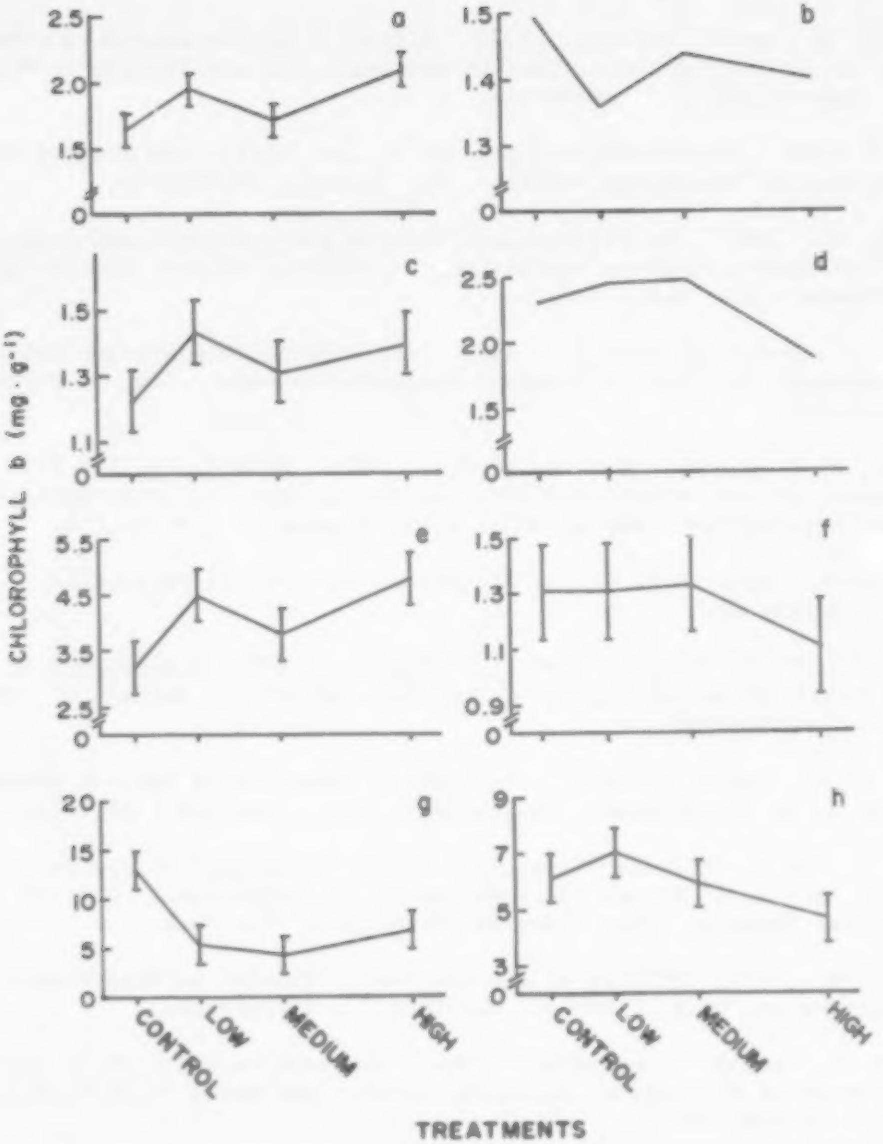

Figure 8.2. Responses of chlorophyll *b* to three concentrations of SO_2 for; a) *Psoralea argophylla*, b) *Sphaeralcea coccinea*, c) *Tragapogon dubius*, d) *Koeleria cristata*, e) *Taraxacum officinale*, f) *Achillea millefolium*, g) *Bromus japonicus*, and h) *Agropyron smithii*. Vertical bars on curves represent least significant ranges (P = 0.05). No bars indicate no significant differences.

REFERENCES

Beckerson, D. W., and G. Hofstra. 1979. Effect of Sulfur Dioxide and Ozone Singly or in Combination on Leaf Chlorophyll, RNA and Protein in White Bean. Can. J. Bot., 57:1940-1945.

Bray, J. R. 1960. The Chlorophyll Content of some Native and Managed Plant Communities in Central Minnesota. Can. J. Bot., 38:313-333.

Brougham, R. W. 1960. The Relationship Between the Critical Leaf Area, Total Chlorophyll Content and Maximum Growth Rate of some Pasture and Crop Plants. Ann. Bot., 24:463-474.

Buttery, B. R., and R. I. Buzzell. 1977. The Relationship Between Chlorophyll Content and Rate of Photosynthesis in Soybeans. Can. J. Plant Sci., 57:1-5.

Heck, W. W., S. V. Krupa, and S. N. Linzon. 1979. Methodology for the Assessment of Air Pollution Effects on Vegetation. APCA Specialty Conference Proceedings. April 19-21, 1978, Minneapolis, Minnesota.

Knabe, W. 1976. Effects of Sulfur Dioxide on Terrestrial Vegetation. Ambio., 5:213-218.

Knudson, L. L., T. W. Tibbets, and G. E. Edwards. 1977. Measurement of Ozone Injury by Determination of Leaf Chlorophyll Concentration. Plant Physiol., 60:606-608.

Lauenroth, W. K., and J. L. Dodd. Chlorophyll Reduction in Western Wheatgrass Exposed to Sulfur Dioxide. (Submitted) Water, Air and Soil Pollut.

Linzon, S. N. 1978. Effects of Airborne Sulfur Pollutants on Plants. pp. 110-162 In: J. O. Nriagu (ed.) Sulfur in the Environment; Part II. Ecological Impacts. John Wiley and Sons, Inc., New York.

Malhotra, S. S. 1977. Effects of Aqueous Sulfur Dioxide on Chlorophyll Destruction in *Pinus contorta*. New Phytol., 78:101-109.

Ovington, J. D., and D. B. Lawrence. 1967. Comparative Chlorophyll and Energy Studies of Prairie, Savannah, Oakwood and Maize Field Ecosystems. Ecology, 48:515-524.

Patterson, D. T., J. A. Bunce, R. S. Alberte, and E. Van Volkenburgh. 1977. Photosynthesis in Relation to Leaf Characteristics of Cotton from Controlled and Field Environments. Plant Physiol., 59:384-387.

Peiser, G. D., and S. F. Yang. 1977. Chlorophyll Destruction by the Bisulfite-Oxygen System. Plant Physiol., 60:277-281.

Rabe, R., and K. H. Kreeb. 1979. Enzyme Activities and Chlorophyll and Protein Content in Plants as Indicators of Air Pollution. Environ. Pollut., 19:119-137.

Sanger, J. E. 1971. Quantitative Investigations of Leaf Pigments from Their Inception in Buds, Through Autumn Coloration, to Decomposition and Falling Leaves. Ecology, 52:1075-1089.

Sestak, Z. 1971. Determination of Chlorophylls *a* and *b*. pp. 672-701, In: Z. Sestak, J. Catsky, and P. G. Jarvis (eds.), Plant Photosynthetic Production: Manual of Methods. Dr. W. Junk, N.V., Publishers, The Hague, 818 pp.

Sokal, R. I., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. 776 pp.

SECTION 9

ADHERENCE OF WATER TO LEAF SURFACES OF *AGROPYRON SMITHII*

J. L. Dodd and J.E. Heasley

ABSTRACT

Leaf blades of live western wheatgrass were immersed in room temperature water to determine an upper limit for water adherence to blade surfaces. A reasonable value for adherence of water to these surfaces was ca. 50 mg · dm^{-2}.

INTRODUCTION

The purpose of this short communication is to document results of a limited investigation designed to determine the approximate maximum quantity of water that will adhere to live external leaf surfaces of *Agropyron smithii* Rydb. Results of the investigation will be incorporated into a mathematical model that simulates the flux of SO_2 to external and internal surfaces of western wheatgrass blades. Since the flux rate of SO_2 to external leaf surfaces varies, in part, with the amount of water adhering to blade surfaces it is necessary to know a maximum value to simulate flux rates immediately following rainfall and dew events.

MATERIALS AND METHODS

We utilized leaf tissue from well watered and fertilized potted plants reared in the greenhouse. Blade portions of leaves were separated into three equal segments -- base, mid-portion, and tips. Each segment varied from 70-90 mm, depending on overall length of blade.

The 32 blade sections of each class were clipped from the living plant, weighed immediately, immersed in distilled water, and reweighed to determine mass of adhered water. Visible droplets of water were shaken from blade segments before reweighing. Length and width of blade segments were then measured to the nearest mm for determination of leaf area. Adhered water was expressed as mg · dm^{-2} (both surfaces).

RESULTS AND DISCUSSION

The basal and mid-portions of live western wheatgrass leaves appear to have a lower capacity to adsorb water than do the tips of leaves (Table 9.1). However, it is possible that this difference is due, at least in part, to underestimating the area of blade tips by assuming that they were triangular in shape. Subsequent examination of other *Agropyron smithii* blades indicated that leaf tips are not always perfectly triangular but are sometimes parallel for a portion of their length and begin to converge to a point within 10-20 mm of the end of the blade.

CONCLUSION

We conclude that the estimates of 58 and 45 mg · dm^{-2} (approximately 50 mg · dm^{-2}) are the most appropriate estimates to be used as an upper limit for the adherence of water to live western wheatgrass blades. This estimate of adherence, of course, does not include water trapped on blades in large droplet form as might occur on *in situ* plants in the field. Water collected on blades in this form would be dependent upon plant morphology, precipitation form, wind speed, and canopy architecture, and falls outside the scope of this limited investigation.

TABLE 9.1. ADHERED WATER ($\bar{X}$ ± SE) ON SURFACES OF LIVE LEAF BLADES OF *Agropyron smithii*

Base	Middle	Tip
mg · dm^{-2}	mg · dm^{-2}	mg · dm^{-2}
58 ± 10	45 ± 11	128 ± 28

SECTION 10

THE INFLUENCE OF PRECIPITATION ON THE SULFUR CONCENTRATION OF *AGROPYRON SMITHII* RYBD. EXPOSED TO SULFUR DIOXIDE

D. G. Milchunas, J. L. Dodd, J. E. Heasley, and W. K. Lauenroth

ABSTRACT

The leaching by rain of SO_2 deposited sulfur from *Agropyron smithii* tillers in a semi-arid Montana grassland exposed to 7.7 pphm SO_2 was estimated to be less than from 5 to 13 percent depending on the time of exposure. Accounting for intensity and frequency of rainfall in addition to duration of SO_2 exposure increased our ability to predict live plant sulfur concentration by 6 percent. Sulfur accumulation in live plants was linear up until the time of senescence. Overwinter losses of plant sulfur were estimated to be 54 and 74 percent for control and SO_2 exposed *Agropyron smithii* tillers, respectively.

INTRODUCTION

The removal of SO_2 from the atmosphere by plants occurs by adsorption onto external leaf surfaces and by absorption via diffusion through the stomata followed by dissolution of the gas in the water film coating the walls of the sub-stomatal chamber. Sulfur dioxide absorbed by the plant can cause disruption of various metabolic processes when the resulting concentrations of sulfur compounds exceed maintenance and growth requirements. Sulfur on the surface of the plant is not involved in plant metabolism.

Studies attempting to distinguish between adsorption and absorption of SO_2 in plants have involved washing or soaking of the exposed plant parts to separate the two fractions. Utilizing plants exposed to $^{35}SO_2$, Garsed and Read (1977) soaked the leaves twice for 45 minutes with gentle shaking to obtain a leachate which contained 15 to 38 percent of the total $^{35}SO_2$. A 20 to 25 percent reduction in sulfur was obtained by Rice *et al.* (1979) for plant parts exposed to SO_2 then placed in a screened jar and rinsed with rapidly flowing tap water for 3 minutes. Values obtained in this manner overestimate surface deposition or even the leaching of sulfur by natural rainfall events.

That rainfall leaches internal substances from plants is well documented, and this topic has been reviewed by Tukey and Tukey (1962) and Tukey (1970). The ease with which internal compounds are leached from plants make it difficult to experimentally discern between absorption and adsorption of SO_2. Data from one study which measured stomatal SO_2 absorption versus total SO_2 deposition to plant leaves showed little adsorption by *Heteromeles arbutifolia* and greater adsorption by *Diplacus aurantiacus* because of it's very sticky leaves (Winner and Mooney, 1980). Considering that; 1) sulfur can accumulate to very high concentrations in plants exposed to SO_2 (Lauenroth *et al.*, 1979), 2) rainfall can leach considerable internal quantities of substances from plants (Tukey, 1970), and 3) sulfur in plant tissues is classified as moderately leachable (Tukey *et al.*, 1958), the amount of sulfur removed by rainfall may be of more biological importance than the commonly used distinction between external and internal sulfur implies.

This study investigated the effect of rainfall on sulfur concentrations of live and dead western wheatgrass (*Agropyron smithii* Rydb.) tillers growing in a native Montana grassland exposed to a controlled level of SO_2.

MATERIALS AND METHODS

Four live and four last year's dead *Agropyron smithii* tillers were collected each morning during July, August, and September from fixed locations on Control and High treatment plots. Daily precipitation was recorded to the nearest tenth of a millimeter at the time of sampling. Total sulfur concentrations of the oven dried (60°C) plant material were assessed with a Leco Induction Furnace (Jones and Isaac, 1972).

Julian date and four variables descriptive of the precipitation events were utilized in stepwise multiple regression analysis to determine their relationships with sulfur content. The four variables used to describe rainfall events were: 1) $Total_5$ = total rainfall in the previous 5 days, 2) #/5 = number of rain days in the previous 5 days, 3) day last = day number of last rain event, and 4) amount last = amount of rain in last rain event.

RESULTS AND DISCUSSION

Precipitation totaling 55 mm occurred on 14 out of 77 days during the course of this study. This was relatively low compared to the 20 year mean of 88 mm for July, August and September in Billings, Montana. The largest rainfall event was 19 mm on 29 July.

Julian date was the best single predictor ($r^2 = 0.68$) of sulfur content for live *Agropyron smithii* tillers exposed to SO_2 (Table 10.1). Accounting for total rainfall in 5 days preceding a sampling date increased prediction. capabilities to 0.70. The use of Julian date and three additional rain event variables increased the coefficient of variation by only 6 percent. No significant relationships were found for sulfur concentrations in SO_2-dead, Control-live, or Control-dead material.

Calculations were made to determine to what degree plant sulfur levels would have had to decline after a rain event in order to have statistically

TABLE 10.1. COEFFICIENTS OF DETERMINATION FOR FIVE VARIABLES UTILIZED TO PREDICT SULFUR CONCENTRATION IN LIVE *Agropyron smithii* PLANTS EXPOSED TO 7.7 PPHM SO_2.*

VARIABLE	r^2
J-date	.6778
J-date, $total_5$	.6961
J-date, #/5	.6804
J-date, day last	.6801
J-date, amount last	.6780
J-date, amount last, $total_5$	.7145
J-date, $total_5$, #/5	.7003
J-date, $total_5$, day last	.6961
J-date, amount last, #/5	.6811
J-date, #/5, day last	.6807
J-date, amount last, $total_5$, #/5	.7361
J-date, amount last, $total_5$, day last	.7324
J-date, $total_5$, #/5, day last	.7024
J-date, amount last, #/5, day last	.6812

* The five variables are Julian date (J-date), the total rainfall in the previous 5 days ($Total_5$), the number of rain days in the previous 5 days (#5), the day number of the last rain event (Day Last), and the amount of rain in the last rain event (Amount Last).

detected a leaching effect. Confidence bands (95 percent) were formulated about the regression of sulfur concentration on date for SO_2-live data. Rain would have had to have leached 13, 5, and 8 percent of the sulfur content of live plants exposed to SO_2 on early, middle, and late sampling dates, respectively, for data points to have fallen outside the 95 percent confidence bands.

Sulfur concentrations in live *Agropyron smithii* tillers on the SO_2 treatment increased through the growing season to a peak in late August; after which they declined (Figure 10.1). A comparison between early and peak sulfur concentration was made by an analysis of variance on the first seven data points and seven consecutive late August data points. Sulfur concentration increased 43 percent ($p = 0.037$) in control-live and 69 percent ($p = 0.001$) in SO_2-live plants. Sulfur concentrations of last year's dead tillers increased 35 and 24 percent ($p = 0.002$ and 0.001) for control and SO_2 treatments, respectively. Estimates of overwintering decreases in sulfur concentration were made by comparing peak live with early season dead sulfur

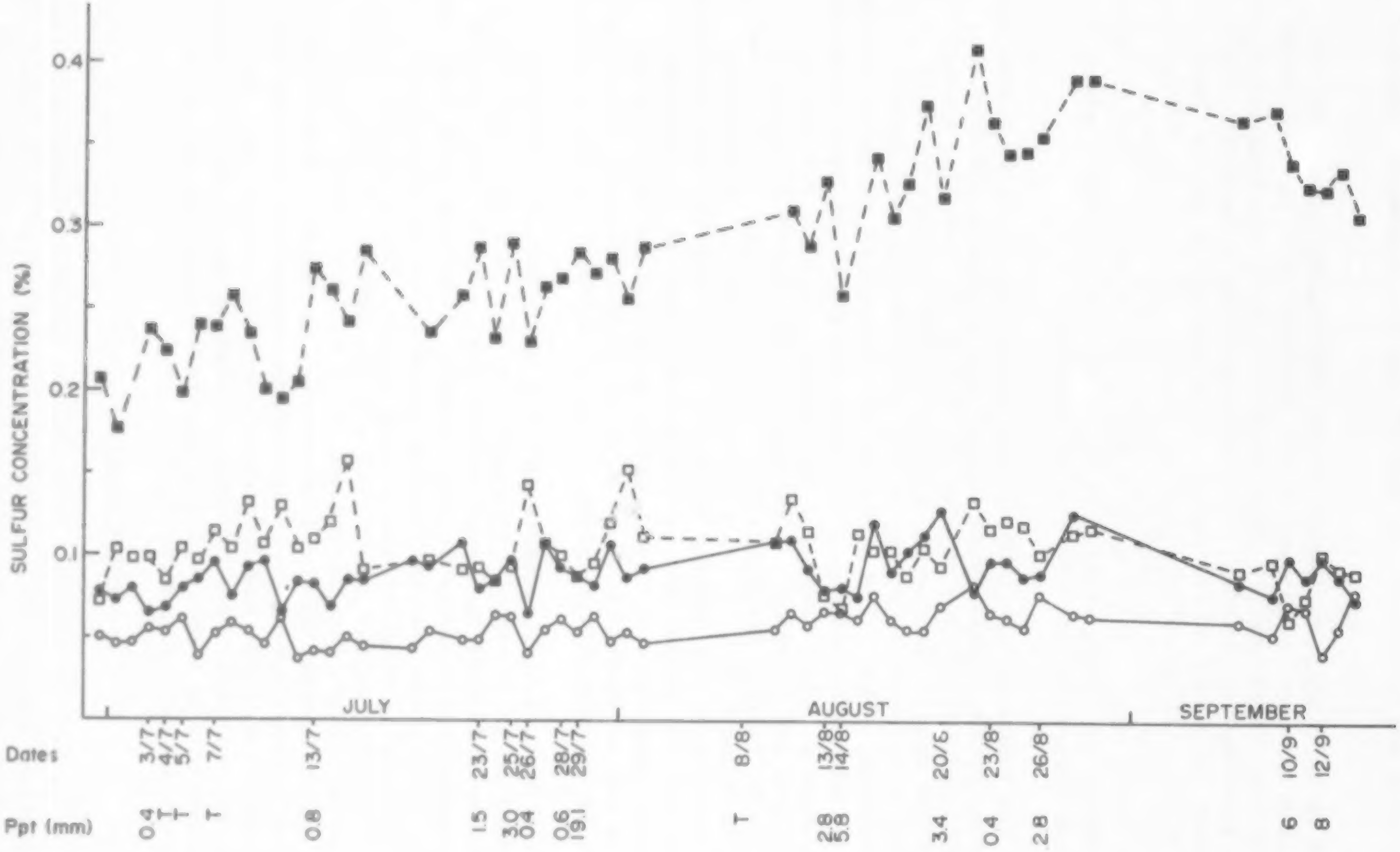

Figure 10.1. Sulfur concentrations in live and previous year *Agropyron smithii* tillers and precipitation (Ppt) for the 1979 growing season. Control live (●——●), Control dead (○——○), SO_2 exposed live (■— —■), SO_2 exposed dead (□— —□).

concentrations. These estimates suggest that overwinter losses were 54 and 74 percent ($p = 0.001$ and 0.036) on the control and SO_2 treatment , respectively.

In addition to leaching, precipitation can potentially affect the distribution of sulfur in a system exposed to SO_2 through its effect on atmospheric SO_2 and by increasing surface deposition because of the high solubility of SO_2 in water (Hocking and Hocking, 1977; Terraglio and Manganelli, 1967). We could not detect increases in the sulfur concentration in ground level dead *Agropyron smithii* tillers after rainfall events. It must be stressed, however, that this study was conducted in a semiarid grassland habitat in a below average rainfall year. In Alberta, Canada, Nyborg *et al.* (1977) reported that rain intercepted by forest trees exposed to SO_2 had a sulfur content 3 to 4 times greater than rain that was not intercepted.

During the wet season in the tropics, the growth and yield of plants may be severely limited by the inability of their roots to absorb and replace nutrients in sufficient quantities to overcome losses via leaching (Tukey, 1970). On the other hand, leaching can be an excretion process by which waste products and substances in excess of requirements can be elimited (Franke, 1967; Stenlid, 1958). The physiological process of guttation through hydathodes and the high salt content of the excreted fluid has been recognized for a long time (Curtis, 1943; Greenhill and Chiball, 1934). The possible function of guttation and leaching in avoidance of SO_2 injury by plants in wet climates warrants further investigation. Guttated fluids can be washed away by rain or be drawn back into the plant (Curtis, 1943). Considering that guttation in dry habitats usually occurs at night when SO_2 concentrations are highest, and that SO_2 deposition is greatly increased on wet surfaces because of the high solubility of SO_2 in water, guttation in dry climates where leaching is not as important of a factor as in wet climates may actually increase SO_2 uptake and constitute another route of entry of sulfur in plants.

Seasonal peak plant sulfur concentration did not coincide with peak plant growth (Dodd *et al.*, 1980). Milchunas *et al.* (In Prep.) observed a peak in *Agropyron smithii* live leaf area in late June - early July, whereas peak sulfur concentrations occurred in late August. Data from this study and Lauenroth *et al.* (1979) indicates that sulfur accumulation in live plants exposed to SO_2 is linear throughout the growing season. The high overwinter loss of 54 and 74 percent sulfur for Control and SO_2 exposed tillers, respectively, occurs as cell walls deteriorate and their contents leach. The following year, sulfur content of the dead material increased. Gosz *et al.* (1976) observed an initial period in litter decomposition when the relative abundance of carbon to minerals was lowered by microbial oxidation. The smaller increase in the sulfur concentration of dead *Agropyron smithii* tillers on the SO_2 treatment may be a reflection of decreased decomposition rates with SO_2 exposure (Dodd and Lauenroth, 1980).

CONCLUSIONS

The leaching by rain of sulfur from *Agropyron smithii* tillers in a semiarid Montana grassland exposed to 7.7 pphm SO_2 was estimated to be less than 5 to 13 percent depending on the duration of exposure. Accounting for the intensity and frequency of rain in addition to the duration of SO_2 exposure increased the ability to predict live plant sulfur concentration by 6 percent. Sulfur accumulation in live plants was linear throughout the growing season ($r^2 = 0.68$). Overwinter losses of plant sulfur were estimated to be 54 and 74 percent for control and SO_2 exposed *Agropyron smithii* tillers, respectively. The following spring, sulfur content of dead *Agropyron smithii* increased through the summer as microbial decomposition reduced organic carbon to mineral ratios.

REFERENCES

Curtis, L. C. 1943. Deleterious Effects of Guttated Fluids on Foliage. Am. J. Bot., 30:778-781.

Dodd, J. L., and W. K. Lauenroth. 1980. Effects of Low-level SO_2 Fumigation on Decomposition of Western Wheatgrass Litter in a Mixed-grass Prairie. In: E. M. Preston and D. W. O'Guinn (eds.) The Bioenvironmental Impact of a Coal-fired Power Plant, Fifth Interim Report, Colstrip, Montana. EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. In Press. pp. 212-215.

Dodd, J. L., W. K. Lauenroth, and R. K. Heitschmidt. 1980. Effects of Controlled SO_2 Exposure on Net Primary Production and Plant Biomass Dynamics. In: E. M. Preston and D. W. O'Guinn (eds.) The Bioenvironmental Impact of a Coal-fired Power Plant, Fifth Interim Report, Colstrip, Montana. EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. In Press. pp. 172-184.

Franke, W. 1967. Mechanisms of Foliar Penetration of Solutions. Ann. Rev. Plant Physiol., 18:281-300.

Garsed, S. G., and D. J. Read. 1977. The Uptake and Metabolism of $^{35}SO_2$ in Plants of Differing Sensitivity to Sulphur Dioxide. Environ. Pollut., 13:173-186.

Gosz, J. R., G. E. Likens, and F. H. Bormann. 1976. Organic Matter and Nutrient Dynamics of the Forest and Forest Floor in the Hubbard Brook Forest. Oecologia, 22:305-320.

Greenhill, A. W., and A. C. Chiball. 1934. The Exudation of Glutamine from Perennial Rye-grass. Biochem. J., 4:1422-1427.

Hocking, D., and M. B. Hocking. 1977. Equilibrium Solubility of Trace Atmospheric Sulfur Dioxide in Water and its Bearing on Air Pollution Injury to Plants. Environ. Pollut., 13:57-64.

Jones, J. B., and R. A. Isaac. 1972. Determination of Sulfur in Plant Material Using a Leco Sulfur Analyzer. J. Agr. Food Chem., 20:1292-1294.

Lauenroth, W. K., C. J. Bicak, and J. L. Dodd. 1979. Sulfur Accumulation in Western Wheatgrass Exposed to Three Controlled SO_2 Concentrations. Plant and Soil, 53:131-136.

Milchunas, D. G., W. K. Lauenroth, and J. L. Dodd. In Prep. Effects of SO_2 Exposure with Nitrogen, and Sulfur Fertilization on the Growth of *Agropyron smithii* Rybd. In: E. M. Preston, D. W. O'Guinn, and R. A. Wilson (eds.) Bioenvironmental Impact of a Coal-fired Power Plant, Sixth Interim Report, Colstrip, Montana. U. S. Environmental Protection Agency, Corvallis, Oregon.

Nyborg, M., J. Crepin, D. Hocking, and J. Baker. 1977. Effect of Sulphur Dioxide on Precipitation and on the Sulphur Content and Acidity of Soils in Alberta, Canada. Water, Air, and Soil Pollut., 7:439-448.

Rice, P. M., L. H. Pye, R. Boldi, J. O'Loughlin, P. C. Tourangeau, and C. C. Gordon. 1979. The Effects of "Low Level SO_2" Exposure on Sulfur Accumulation and Various Plant Life Responses of Some Major Grassland Species on the ZAPS Sites. In: E. M. Preston and T. L. Gullett (eds.) Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 494-591.

Stenlid, G. 1958. Salt Losses and Redistribution of Salts in Higher Plants. Encycl. Plant Physiol., 4:615-637.

Terraglio, F. P., and R. M. Manganelli. 1967. The Absorption of Atmospheric Sulfur Dioxide by Water Solutions. J. Air Pollut. Control Assc., 17:403-406.

Tukey, Jr., H. B. 1970. The Leaching of Substances from Plants. Ann. Rev. Plant Physiol., 21:305-324.

Tukey, Jr., H. B., and H. B. Tukey, Sr. 1962. The Loss of Organic and Inorganic Materials by Leaching from Leaves and Other Above-ground Plant Parts. In: Radioisotopes in Soil-Plant Nutrition Studies, Proceedings IAEA, Vienna. p. 289-302.

Tukey, Jr., H. B., H. B. Tukey, Sr., and S. H. Wittwer. 1958. Loss of Nutrients by Foliar Leaching as Determined by Radioisotopes. Proc. Am. Soc. Hort. Sci., 71:496-506.

Winner, W. E. and H. A. Mooney. 1980. Ecology of SO_2 Resistance: II. Photosynthetic Changes of Shrubs in Relation to SO_2 Absorption and Stomatal Behavior. Oecologia, 44:296-302.

SECTION 11

SULFUR DISTRIBUTION AND ALLOCATION IN WESTERN WHEATGRASS EXPOSED TO SO_2 UNDER VARIABLE NUTRIENT SULFUR REGIMES

C. J. Bicak, W. K. Lauenroth, and J. L. Dodd

ABSTRACT

Sulfur concentrations in western wheatgrass plants were measured in shoots and roots, individual leaves, and leaves subdivided into three blade segments. Plants were exposed to Control or High SO_2 and were maintained in hydroponic solutions without sulfur, and at both optimum (2 mM) and double the optimum (4 mM) concentration of sulfur. Leaf tips contained more sulfur than middle or basal blade segments and fumigated leaves contained more sulfur than unfumigated leaves. Added SO_2 did not alter the sulfur contents of either shoots or roots. However, added sulfur in the nutrient medium increased root and shoot sulfur levels. Shoot-root biomass ratios were greater in fumigated plants in the early season. Late in the season however, differences due to SO_2 fumigation were not discernible.

INTRODUCTION

With the rapid development of coal mining and associated coal-fired power plants in the western states, the impact of air pollutants on grassland ecosystems has become of considerable importance (Preston and Gullett, 1979). Cool season grasses make up the predominant vegetation cover of prairies in coal-producing regions of southeast Montana and are an important component of the diet of both wild and domestic herbivores. While many investigations have centered upon plant responses to SO_2 under laboratory conditions, few studies have utilized field exposures. Laboratory fumigation trials rarely simulate ambient fluctuations in SO_2 concentration and present problems in extrapolation to field conditions. The objective of this study was to determine the interactive effects of atmospheric and substrate S on sulfur metabolism of a C_3 grass native to mixed prairies in southeast Montana. Emphasis was placed upon sulfur distribution and allocation in western wheatgrass as well as alterations in the relative masses of shoots and roots.

Many reviews have recognized sulfur dioxide injury to vegetation (Jacobson and Hill, 1970; Wolozin and Landau, 1966), but researchers have only recently begun to examine the physiological alterations and the subsequent fate of SO_2 in the plant system (Malhotra and Hocking, 1976; Ziegler, 1975). Sulfur distribution and allocation in native plants have not been fully characterized and the nutrient has often been considered relatively immobile once an optimal leaf sink concentration has been achieved (Salisbury and Ross, 1978; Laüchli, 1972). Optimal sulfur concentrations have been suggested to range from 1500 $\mu g \cdot g^{-1}$ to 3000 $\mu g \cdot g^{-1}$ in forage grasses (Metson, 1973). Plants in an atmosphere containing measurable levels of SO_2 may be subjected to a physiological stress as the sulfur concentration in the tissue approaches some toxic level. Tingey *et al.* (1978) found that western wheatgrass was relatively resistant to SO_2 with concentrations greater than 100 pphm required to induce visible injury. Even though visible injury may be minimal, sulfur levels greater or less than an optimal level may induce severe physiological stress upon a plant. Malhotra and Hocking (1976) have reviewed current physiological literature and have noted inhibitory effects of SO_2 on plants that include interference with photosynthetic CO_2 fixation and a reduction in the buffering capacity of plant cells. It is conceivable then, that such subtle shifts in whole plant sulfur status may imply long-term alterations in the mixed-prairie ecology.

Plant sulfur content and partitioning are not well characterized under SO_2 fumigation conditions in the field. The focus of this paper is upon this problem. In light of the foregoing discussion the following objectives have been established in this study; 1) determine the role of deficient and excess nutrient sulfur levels in conjunction with SO_2 in affecting changes in sulfur distribution and accumulation in western wheatgrass, 2) determine the relative mobility of sulfur in western wheatgrass, 3) determine potential plant tissue sinks for sulfur.

MATERIALS AND METHODS

Experimental Design

To provide a nutrient system that would allow precise manipulation of sulfur concentration, hydroponic containers were employed. Extensive research has been conducted with hydroponic systems and cultivated species such as tomatoes and cucumbers (Salisbury and Ross, 1978) while concern for native vegetation has been limited. Although some work with native grasses and nutrient solutions has been reported in the literature (Williams and Kemp, 1978; Detling *et al.*, 1979) most of this has focused upon photosynthetic responses to changing environmental conditions.

Western wheatgrass (*Agropyron smithii* Rybd.) plants were grown in hydroponic culture with a full complement of elemental nutrients in one-seventh strength solution as described by Hoagland and Arnon (1938). Containers held approximately 8 liters and adequately sustained 24 plants. Upon transfer from the greenhouse to the fumigation site in Montana, plants were moved to 4 liter styrofoam containers with eight plants per container. Containers were covered and taped to minimize deposition of SO_2 to solution surfaces.

Two experimental periods were selected during the growing season. The first progressed from June 20 to June 26, 1979 (147 hours) while the second progressed from August 12 to August 18, 1979 (147 hours). These time intervals coincided roughly with periods of maximal photosynthetic rates and 50 percent senescence respectively, in native western wheatgrass plants *in situ*. Developmental stages were similar in greenhouse-grown and *in situ* plants in the spring. In the early season, four experimental containers were placed on each of the Control and SO_2-fumigated plots. Nutrient sulfur concentrations were varied such that one container received no sulfur, one received complete sulfur (2 mM), and one received double (4 mM) the complete sulfur complement. A fourth container held eight dry western wheatgrass plants. These plants were not physiologically functional and were included in the design to simulate senescent leaf material in which the predominant sulfur input would be surface deposition. The late season trial was similar in design to the early season trial but greenhouse-grown plants were in an earlier developmental stage than *in situ* plants.

Plant Analysis

Plant material was oven-dried at 60°C to a constant weight. Sulfur content of leaves, shoots, and roots was assessed in all plant material in hydroponic containers across all SO_2 fumigation plots. At the time of harvest, plants were divided into shoot and root components to prevent potential subsequent sulfur translocation. Some individual blades were further divided into leaf blade tips, midsections, and bases each approximately 6 cm long. Sulfur content (mg S/mg tissue) was measured with a LECO Induction Furnace (Laboratory Equipment Corporation, St. Joseph, Michigan, USA) with the inorganic sulfate ion ($SO_4^{=}$) the primary form of sulfur recovered. Data were subjected to analyses of variance to discern any treatment differences while means reported include standard errors about them.

RESULTS AND DISCUSSION

Most gaseous pollutant investigations have focused upon responses of dicots, particularly cultivated species (Jacobson and Hill, 1970; Guderian, 1977). Since the growth and development of grasses are notably different (Esau, 1965), extrapolation of responses to air pollutants from one form of growth to another may not be valid. Development of dicots is characterized by leaf expansion in the light, whereas a specialized leaf unrolling process occurs in grasses (Leopold and Kriedemann, 1975) which results in a gradient in the functional age of individual grass blades. Development of grasses proceeds from the crown with older leaves near the base and younger leaves near the tip of the plant. Individual leaf initiation and expansion occurs such that the leaf tip is the oldest and the base the youngest portion of the leaf (Langer, 1972). Since there is a difference in the development of grasses and dicotyledonous species it may be assumed that patterns in plant sulfur content and distribution may also be very different. To better understand the impact of coal-fired power plants upon mixed-prairie vegetation, research efforts must concentrate upon endogenous grass species. This paper focuses upon comparisons of plant material exposed under control conditions ($-SO_2$) and with SO_2 fumigation (7.7 pphm) as well as comparisons between plants grown in different S-nutrient solutions. Sulfur distribution and allocation are discussed below with consideration of three levels of organization; 1) segments of leaf blades, 2) whole leaves, and 3) whole plants.

Leaf Segments

Sulfur content in segments of the leaf blade was significantly different ($P \leq 0.01$) in both the early and late exposure periods of the growing season with the highest sulfur levels in the oldest portion of the grass blade (tip) and the lowest sulfur levels in the youngest portion of the blade (base). The gradient in blade sulfur content from oldest to youngest tissues was consistent with and without SO_2 fumigation (Figure 11.1). While there was no significant interaction between SO_2 exposure level and the sulfur content of individual parts of the blade, the data and the evidence of Lauenroth *et al.* (1980) lead us to believe that sulfur levels were higher in leaf blade parts from unfumigated plants than from fumigated plants. Gradients in sulfur content between tips and bases were also reported by Jäger (1976), utilizing spruce needles exposed to SO_2. Guderian (1977) suggested the secondary transport of sulfur taken up as SO_2 to blade tips and margins as an explanation for this gradient. Although Jäger (1976) and Guderian (1977) both cite Halbwachs (1963) to explain this transport via the movement of sulfate in water and an associated water potential gradient toward tips and margins, it is conceivable that tips are simply exposed for a longer period of time and accumulate more sulfur than the younger portion of the blade. Lower sulfur levels in parts of the blade from fumigated plants suggest that a translocation mechanism may be implicated in plants exposed to SO_2 that shunts sulfur from

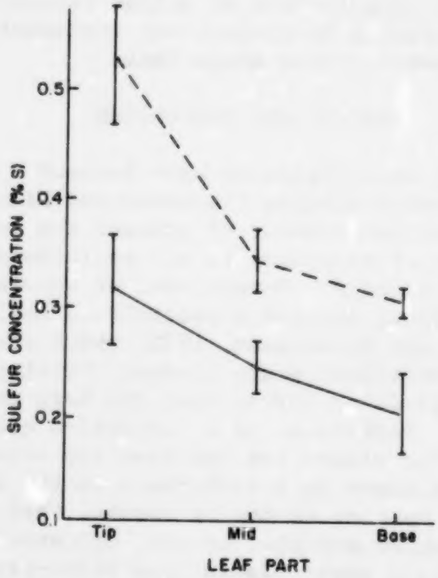

Figure 11.1. Sulfur content in segments of the leaf blade following late season exposure. ------ ($- SO_2$) ——— ($+ SO_2$).

foliar parts to roots more readily than when the ambient atmospheric sulfur levels are negligible. That is, the sulfate sulfur concentration gradient between shoots and roots is steepened. Such a transfer however, does not necessarily imply an increase in biomass (Table 11.1). According to Münch (1926), solute concentration at a source (*i.e.*, shoot) may dictate the rate and bulk flow of that solute to a sink (*i.e.*, root). Ionic transfer may be similar as downward movement of sulfate sulfur through phloem tissue is accelerated. Rennenberg *et al.* (1979), working with tobacco, presented evidence that reduced sulfur, with SO_2 as its source (Garsed and Read, 1977), is translocated from leaves to roots.

Differences in the sulfur content of segments of leaf blades were also related to variations in nutrient solution sulfur (Figure 11.2). Sulfur content varied only with blade segment ($P \leq 0.01$) and not sulfur nutrition with results similar with and without SO_2 fumigation. Although the effects of nutrient solution treatments upon the sulfur content of parts of the blade were not significantly different from one another, the trend suggests consistently greater blade segment sulfur content when the nutrient solution contains twice the optimal level. These data also support the idea of a leaf tip to base gradient in sulfur concentration. Differences in sulfur content among the three nutrient solutions were smallest in leaf bases. Accumulation of sulfur in plant material kept in high sulfur nutrient solutions may be explained by a more traditional translocation mechanism than may operate when SO_2 is a primary controller or regulator of sulfur distribution. Sulfate is

TABLE 11.1. SHOOT-ROOT BIOMASS RATIOS FOR WESTERN WHEATGRASS PLANTS WITH AND WITHOUT SO_2 AND WITH THREE NUTRIENT SOLUTION SULFUR LEVELS *

Early Season (s/r)			Late Season (s/r)		
† A no S	1.13	No difference	A no S	1.62	No difference
D no S	1.83	(P = 0.12)	D no S	2.16	(P = 0.17)
A comp S	2.14	No difference	A comp S	2.39	No difference
D comp S	2.21	(P = 0.41)	D comp S	2.21	(P = 0.41)
A doub S	1.48	No difference	A doub S	1.60	No difference
D doub S	1.63	(P = 0.35)	D doub S	2.38	(P = 0.14)
A dep	0.86	No difference	A dep	1.26	No difference
D dep	0.88	(P = 0.47)	D dep	0.78	(P = 0.12)

* Two sample T-tests indicated no significant difference ($P \leq 0.05$) between the two fumigation plots among four nutrient solution treatments, across two time periods in the growing season.

† A = ($- SO_2$)
D = ($+ SO_2$)

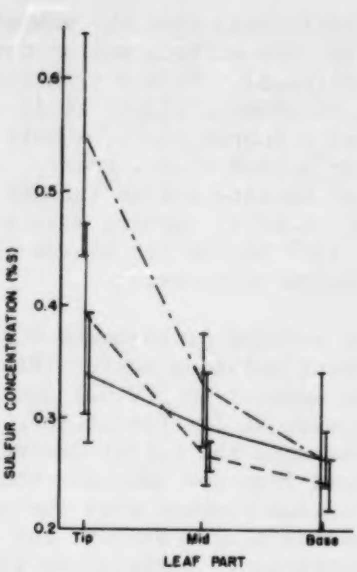

Figure 11.2. Sulfur content in segments of the leaf blade following late season exposure. ------ No S solution —— Complete S solution - —— - Double S solution.

translocated from roots to the leaves through the transpiration stream. In this way the excess sulfur load to which roots are exposed, may be alleviated. Early and late season data were similar.

Whole Leaves

Statistical analysis of the sulfur content of whole leaves indicated significantly ($P \leq 0.05$) greater sulfur in leaves exposed to 7.7 pphm SO_2 than in unexposed plants for all the nutrient solution treatments in the early season (Figure 11.3). In contrast to this, sulfur content in whole leaves of fumigated plants across the nutrient solution treatments was significantly less ($P \leq 0.05$) than with no fumigation in the late season with difference due primarily to enhanced sulfur levels with the complete sulfur solution (Figure 11.4). To examine this apparent anomaly in more detail, data were subjected to a three way analysis of variance, including each of four leaf ages, with the SO_2 and nutrient solution sulfur information. Differences in sulfur content were not significant among leaves of different developmental or physiological ages. We suggest that the 147 hour exposure interval is insufficient in both the early and late periods of the season to identify trends in plant sulfur distribution for all leaves combined or on an individual leaf age basis.

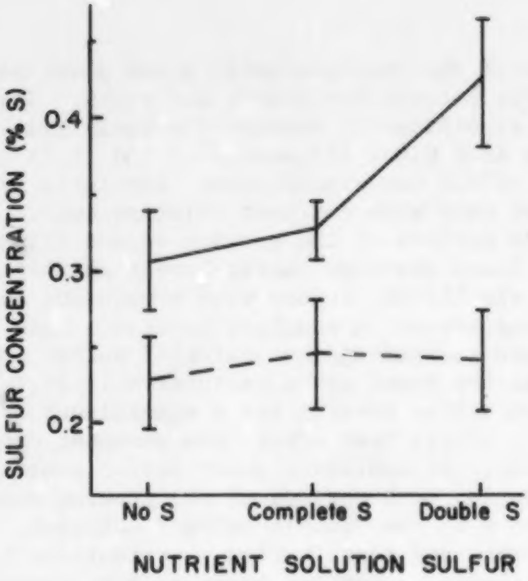

Figure 11.3. Sulfur content in whole leaves following early season exposure. ------ (- SO_2) ——— (+ SO_2).

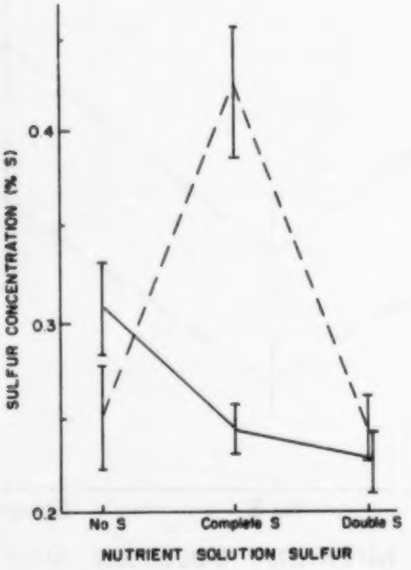

Figure 11.4. Sulfur content in whole leaves following late season exposure. ------ (- SO_2) ——— (+ SO_2).

Whole Plants

A final analysis of the hydroponically grown plant material involved determination of sulfur content for shoots and roots. Shoot and root sulfur content did not vary significantly between the early and late season periods in unfumigated plants ($P \leq 0.05$) (Figures 11.5 and 11.7) while the influence of nutrient solution sulfur was significant. Similarly, shoot and root sulfur contents varied only with nutrient solution sulfur and not the SO_2 fumigation during both periods of the growing season (Figures 11.6 and 11.8). Linzon *et al.* (1979) found elevated sulfur levels in white birch foliage and associated low level visible SO_2 injury with a mean SO_2 concentration of 1.1 pphm during the growing season in southern Ontario. Lack of difference between unfumigated and fumigated plant material sulfur content in our study may be attributable to the short exposure interval. It is noteworthy, however, that nutrient solution sulfur content had a significant effect ($P \leq 0.05$) on shoot and root sulfur, albeit that effect was somewhat different in the early and late season periods. In addition, shoot sulfur content was greater than root sulfur content during both periods of the growing season with the only exception occurring on A in the "double sulfur" solution. That sulfur content of dried, inactive shoots and roots varied, particularly in the early season, is not readily reconcilable. Perhaps shoot sulfur content is always greater

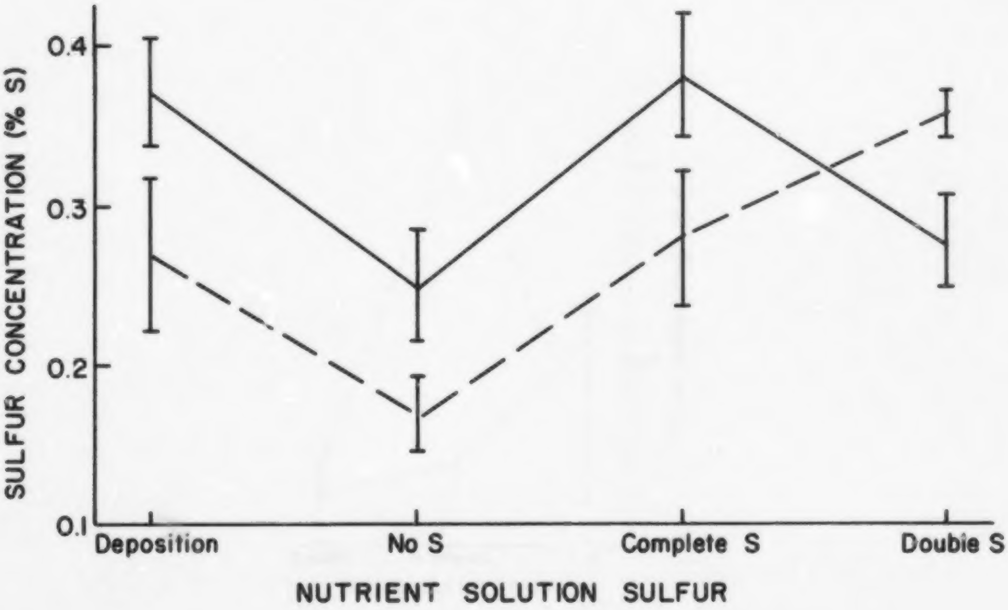

Figure 11.5. Sulfur content in shoots and roots following early season exposure (- SO_2). ------ roots —— shoots.

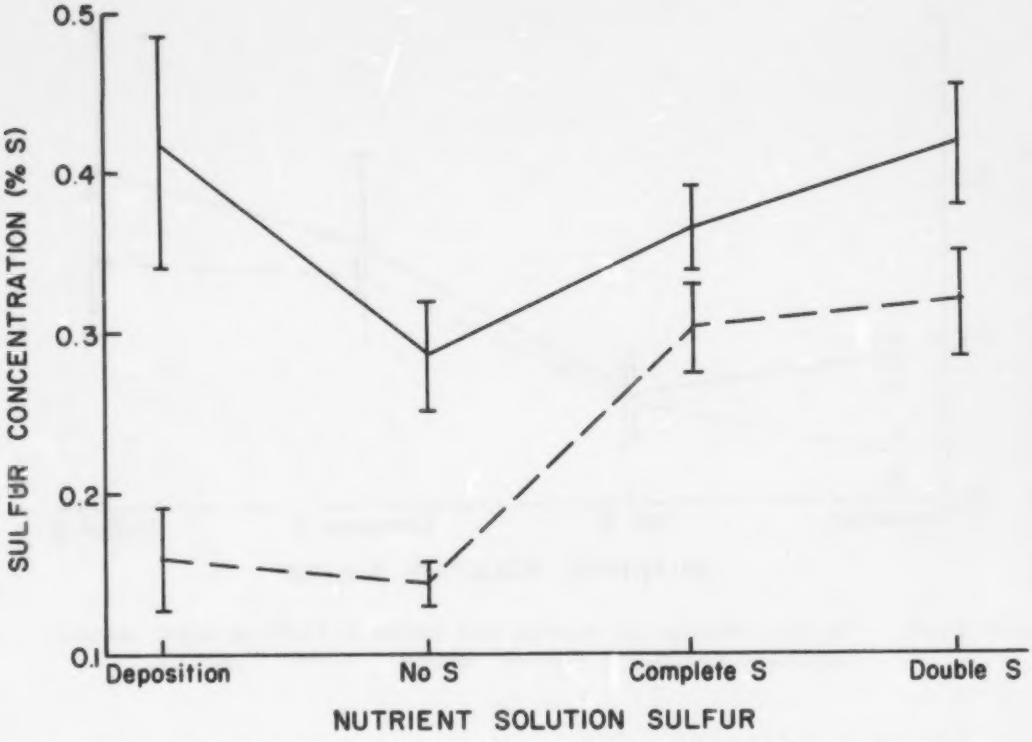

Figure 11.6. Sulfur content in shoots and roots following early season exposure (+ SO_2). ------ roots ——— shoots.

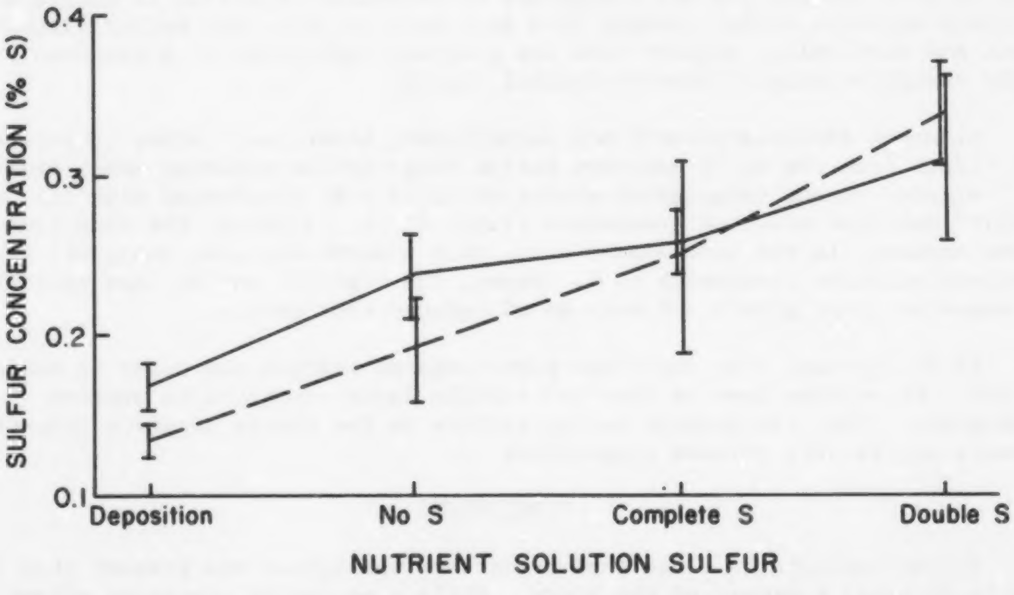

Figure 11.7. Sulfur content in shoots and roots following late season exposure (- SO_2). ------ roots ——— shoots.

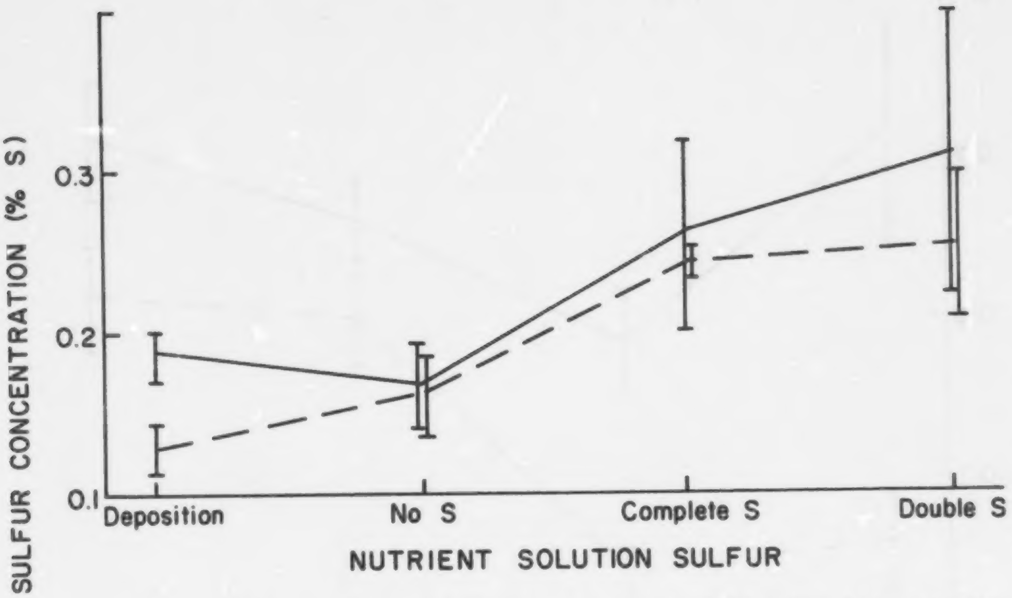

Figure 11.8. Sulfur content in shoots and roots following late season exposure (+ SO_2). ------ roots ——— shoots.

than root sulfur content with any additional sulfur accumulation in SO_2 exposure simply due to deposition upon the dried shoots. In general, Figures 11.5 to 11.8 express the not unexpected accumulation of sulfur in plants as nutrient solution sulfur changes from near zero to twice the optimal level. Shoot and root sulfur content data are probably indicative of a response rapid enough to suggest true biological trends.

Although differences were not significant, shoot-root ratios calculated for plants from the early exposure period suggested an enhanced shoot growth in fumigated versus unfumigated plants which is most pronounced with the "no sulfur" nutrient solution treatments (Table 11.1). Although the same trend seems apparent in the late season data, root biomass was less among all the nutrient solution treatments on D. Hence, the high s/r may be less indicative of enhanced shoot growth and more so of reduced root growth.

It is apparent that important physiological changes may occur in response to SO_2. At no time have we observed visible injury symptoms in western wheatgrass. Yet, the altered sulfur balance in the plants suggests long-term effects of, as yet, unknown proportions.

CONCLUSIONS

Sulfur contents in blade tips of western wheatgrass was greater than in middle or basal segments of the blade. While a secondary transport scheme has been suggested to account for the movement of sulfate to blade tips and margins in the transpirational stream, it is conceivable that tips are simply exposed for a longer period of time than the lower, younger portion of the blade.

The duration of the exposure periods (147 hours) appeared inadequate to discern any sulfur distribution trends among whole leaves yet, fumigated leaves contained more sulfur than unfumigated leaves in the early season trials.

Sulfur dioxide treatments had no effect on shoot and root sulfur content but the effect of nutrient solution was significant. Shoot sulfur content was greater than root sulfur content during both periods of the growing season with the only exception occurring on the unfumigated control in the "double sulfur" solution.

Trends suggest that shoot-root ratios were greater in the early season on the plot fumigated with SO_2 but the trend late in the season suggested reduced root growth rather than increased shoot growth. Further controlled exposure studies are required to document consistent reductions in root growth in the late season.

REFERENCES

Detling, J. K., M. I. Dyer, and D. T. Winn. 1979. Net Photosynthesis, Root Respiration, and Regrowth of *Bouteloua gracilis* Following Simulated Grazing. Oecologia (Berl.), 41:127-134.

Esau, K. 1965. Plant Anatomy. John Wiley and Sons, Inc., New York. 767 pp.

Garsed, S. G., and D. J. Read. 1977. The Uptake and Metabolism of $^{35}SO_2$ in Plants of Differing Sensitivity to Sulphur Dioxide. J. Environ. Pollut., 13:173-186.

Guderian, R. 1977. Air Pollution. Springer-Verlag, Berlin. 127 pp.

Halbwachs, G. 1963. Utersuchnunger über gerichete aktive strömungen and strofftransporte in blatt. Flora, 153:333-357.

Hoagland, Dr. R., and D. I. Arnon. 1938. University of California Agricultural Experimental Station Circular no. 347.

Jacobson, J. S., and A. C. Hill, eds. 1970. Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas. Air Pollut. Control Assoc., Pittsburgh, Pennsylvania.

Jäger, H. J. 1976. S-Lokalisation in SO_2 begasten Fichtennadeln. Eur. J. For. Pathol., 6:25-29.

Langer, R. H. M. 1972. How Grasses Grow. Edward Arnold Publishers, London.

Laüchli, A. 1972. Translocation of Inorganic Solutes. Ann. Rev. Plant Physiol., 23:197-218.

Lauenroth, W. K., C. J. Bicak, and J. L. Dodd. 1980. Sulfur Accumulation in Western Wheatgrass Exposed to Three Controlled SO_2 Concentrations. In: Bioenvironmental Impact of a Coal-fired Power Plant. Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn (eds.) EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 147-152.

Leopold, A. C., and P. E. Kriedemann. 1975. Plant Growth and Development. McGraw-Hill Book Company.

Linzon, S. M., P. J. Temple, and R. G. Pearson. 1979. Sulfur Concentrations in Plant Foliage and Related Effects. J. Air Pollut. Control Assoc., 29:520-525.

Malhotra, S. S., and D. Hocking. 1976. Biochemical and Cytological Effects of Sulfur Dioxide on Plant Metabolism. New Phytol., 76:227-237.

Metson, A. S. 1973. Sulphur in Forage Crops; Plant Analysis as a Guide to the Sulfur Status of Forage Grasses and Legumes. Tech. Bull. No. 20, The Sulphur Institute, Washington, D.C. 24 pp.

Münch, E. 1926. Die Stoffbewegungen in der Pflanze [Translocation in plants]. Fischer, Jena.

Preston, E. M., and T. L. Gullett. 1979. Spatial Variations of Sulfur Dioxide Concentrations on ZAPS During the 1977 Field Season. In: Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett (eds.) EPA-600/3-78-021. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 306-326.

Rennenberg, H., Schmitz, and L. Bergmann. 1979. Long-distance Transport of Sulfur in *Nicotiana tabacum*. Planta., 147:57-62.

Salisbury, F. B., and C. W. Ross. 1978. Plant Physiology. Wadsworth Publishing Company. Belmont, California.

Tingey, D. T., L. Bard, and R. W. Field. 1978. The Relative Sensitivity of Selected Plant Species to Several Pollutants Singly and in Combination. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Third Interim Report, Colstrip, Montana. E. M. Preston and R. A. Lewis (eds.) EPA-600/3-78-021. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 519-522.

Williams, G. J., and P. R. Kemp. 1978. Simultaneous Measurement of Leaf and Root Gas Exchange of Shortgrass Prairie Species. Bot. Gaz., 139:150-157.

Wolozin, H., and E. Landau. 1966. Crop Damage from Sulphur Dioxide. J. of Farm Economics, 394-405.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79-105.

SECTION 12

EFFECTS OF SO_2 EXPOSURE WITH NITROGEN AND SULFUR FERTILIZATION ON THE GROWTH OF *AGROPYRON SMITHII* RYDB.

D. G. Milchunas, W. K. Lauenroth, J. L. Dodd, and T. J. McNary

ABSTRACT

Exposure of western wheatgrass (*Agropyron smithii* Rydb.) to 3.0 pphm SO_2 during the growing season resulted in increased leaf areas, while at 5.4 and 9.1 pphm SO_2 leaf area declined to near Control levels. Nitrogen fertilization increased leaf areas with increasing levels of SO_2 indicating that N fertilization can ameliorate the effects of SO_2 pollution. $SO_2 \times N$ interactions also resulted in a shift in phenological development. Nitrogen fertilized plants exhibited an increased rate of senescence and time of senescence for 9.1 compared to 3.0 or 5.4 pphm SO_2, but only a more rapid rate of senescence compared to the Control. Nitrogen fertilization without SO_2 also resulted in a more rapid rate of senescence. Differentiating between rate and time of senescence is necessary when examining the effects of any compound which can be both a fertilizer and a toxic substance. No $SO_2 \times SO_4$ interaction was observed.

INTRODUCTION

Sulfur dioxide is the most widespread air pollutant that is capable of causing severe injury and damage to vegetation (Saunders and Wood, 1973; Stern, 1968). Plants vary greatly in their response to SO_2 (Guderian and Van Haut, 1970). This variation is due to their genetic composition, to their response to environmental factors, to their nutritional status, and to the time and concentration of exposure to SO_2 (Guderian, 1977). Sulfur dioxide can result in the general disruption of photosynthesis, respiration and other fundamental cellular processes and thereby cause reduction in plant growth and yield (Ziegler, 1975). On the other hand, sulfur is necessary for the general metabolism of plants because it is a major component of amino acids, certain vitamins, glutothione, coenzyme A, and also functions in the activation of certain proteolytic enzymes, in the formation of glucoside oils, and of certain disulfide linkages that have been associated with the structural characteristics of protoplasm (Ziegler, 1975). Because of the nutritional/ phytotoxic effects of sulfur and the differences in species response, contradictory results can be found in the literature with respect to plant growth and yield as affected by atmospheric SO_2, soil sulfate, and the interaction between the two sulfur sources.

Cowling and Lockyer (1978) demonstrated that exposure of sulfur-deficient perennial ryegrass to SO_2 at low concentration (2 pphm) can correct sulfur deficiency. Additionally, they found no adverse effects of SO_2 on plants supplied with adequate sulfur through the soil. In contrast to this, Bell and Clough (1973) measured 50 percent reduction in yield of S 23 perennial ryegrass, at concentrations of 7.3 pphm SO_2. Faller (1971) reported that 57.7 pphm SO_2 had a favorable effect on tobacco leaf development even when sufficient sulfate (0, 40, 80 and 240 ppm) was available from the substrate. They further concluded that SO_2 was equivalent to sulfate as a source of sulfur for plant growth. Thomas *et al.* (1943) found 8.5 pphm SO_2 less efficient than sulfate and yield of fumigated plots with various amounts of sulfate (0, 0.8, 1.5, and 10 ppm) to be the same as yield of non-fumigated checks. Katz (1949), Setterstrom *et al.* (1938), Booth *et al.* (1976), Brisley and Jones (1950), Brisley *et al.* (1959), Daines (1968), Thomas *et al.* (1944) reported that crop yields were not significantly reduced by SO_2 when visible injury symptoms were absent. Guderian and Stratmann (1962), Lockyer *et al.* (1976), and Tingey *et al.* (1971) demonstrated a suppression of plant growth by SO_2 with no visible leaf necrosis. Lockyer *et al.* (1976) observed a reduction in yield of perennial ryegrass grown without addition of sulfate at low SO_2 concentration and a reduction in yield of plants grown in 15.4 pphm SO_2 irrespective of the addition of sulfate to the soil. Leone and Brennan (1972) found SO_2 injury was more pronounced in plants grown at higher sulfate levels. Eaton *et al.* (1971) observed no reduction in growth of cotton and tomatoes exposed to SO_2 (7.7 pphm) but a 37 and 54 percent growth reduction, respectively, upon the addition of sulfate salts.

In plant and animal nutrition, sulfur metabolism is closely related to that of nitrogen. Sulfur and nitrogen are utilized in growth according to a stoichiometric relation which closely agrees with the elementary composition of protein, the principal consumer of these elements (Dijkshoorn *et al.*, 1960). Aulakh *et al.* (1976) and Pumphrey and Moore (1965) reported maximum yield for alfalfa with a total N:total S ratio of 11:1. Increased sulfate application improved N uptake and enhanced protein production (Aulakh *et al.*, 1976). Further, evidence is accumulating that certain sulfur-containing enzymes perform a vital role in interconversion of nitrogenous compounds (Rendig and McComb, 1959). Medvedev (1957) has postulated that sulfhydryl groups, through their effect on protein structure, regulate to some degree the course of ontogenetic development. Because roots apparently have the ability to reduce sufficient sulfate to provide for their own needs but do not translocate appreciable amounts of reduced sulfur to the shoots (Salesbury and Ross, 1969), the increased supply of readily available sulfur from low levels of SO_2 exposure may actually enhance protein production and plant growth (Ziegler, 1975).

High levels of SO_2 fumigation (59.2 pphm) have been shown to reduce protein synthesis (Godzik and Linskens, 1974). Constantiniodou and Kozlowski

(1979) reported a significant reduction in protein and total nonstructural carbohydrate when *Ulmus americana* seedlings were fumigated with high levels of SO_2. The reduction in protein was accompanied by increases in free amino acids (Godzik and Linskens, 1974). Steinberg *et al.* (1950) proposed that the chlorosis commonly shown by plants subjected to nutritional stress is accompanied by an accumulation of free amino acids.

There is a need for further investigation of plant response to SO_2 × $SO_4^=$ × N because 1) there are contradictory reports on SO_2 × $SO_4^=$ interactions, 2) very little information is available on SO_2 × N interactions, and 3) there is especially a lack of information in this regard for native rangeland species grown under field conditions. Under natural conditions, changes in humidity, light intensity, temperature (Ziegler, 1975), dew, mist, and rain (Garsed and Read, 1977; Malhotra and Hocking, 1976) can affect plant uptake of SO_2. This study investigated the effect of three levels of SO_2 fumigation and sulfur, nitrogen and sulfur plus nitrogen fertilization on the growth of western wheatgrass (*Agropyron smithii* Rydb.) in a natural grassland ecosystem.

MATERIALS AND METHODS

The study area was located on the divide between the Powder and Tongue River drainage basins in Custer National Forest, Montana (45°15'N, 106°E). A split plot design was used with SO_2 treatments as the main plots and fertilizer treatments as the split plots. The objectives of the SO_2 treatments were to maintain 30-day median SO_2 concentrations of zero (Control), 2 (Low), 5 (Medium), and 10 pphm (High) throughout the April to October growing season. Each 0.52-ha treatment plot was subdivided into two replicates. Sulfur dioxide was delivered to the treatment plots through a network of aluminum pipes located approximately 0.75 m above the ground surface. Concentrations were monitored with a Meloy Laboratories (Model SA 160-2) sulfur analyzer through teflon lines located within the plant canopy. Analysis of the 1978 monitoring data resulted in SO_2 growing season means of <1.0, 3.0, 5.4, and 9.1 pphm for the Control, Low, Medium and High treatments, respectively. Geometric mean concentrations of SO_2 during daylight hours were one-third less than the 24-hour day values reported above.

Four 1 × 2 m contiguous fertilizer plots were located in each replicate for each SO_2 treatment. Fertilizer treatments were: Control, nitrogen (150 kg nitrogen/ha as ammonium nitrate), sulfur (15 kg sulfur/ha as magnesium sulfate), and nitrogen plus sulfur (150 kg nitrogen plus 15 kg sulfur/ha). The treatments were applied in solution on April 15, 1978. The amount of water used in application of the fertilizers (equivalent to 5 mm rainfall) was also applied to the Control plots. Soils on the study area were classified as Farland silty clay loams. Soil N and $SO_4^=$ levels for the A-11 horizons from soil pits adjacent to the fumigated plots averaged 0.1 percent N and 0.1 meq. $SO_4^= \cdot 100\ g^{-1}$ soil.

Ten *Agropyron smithii* plants on each fertilizer treatment-replicate were marked by placing a large metal washer over the plant (*i.e.*, 20 plants per fertilization treatment per SO_2 treatment). Growth of each plant was assessed by counting the number of leaves and measuring, to the nearest mm, plant height, green and brown length of each leaf blade and the maximum width of the green and brown portions. Each leaf blade was assumed to be triangular for leaf area calculations. Live and dead leaf areas were calculated separately. Decreases in the area of individual leaves between sample dates were assumed to represent leaf material added to the litter.

Analysis of variance was performed using a split-split plot design with the following breakdown: main plot-SO_2 level; split plot - a N × S two by two factorial; split-split plot-sample date. Replicates were viewed as a randomized block due to positioning. Tukey's Q values were utilized to compute least significant ranges (LSR) and identify significant differences between means (Sokal and Rohlf, 1969).

RESULTS

Significant sample date × SO_2 ($P = 0.055$) and date × nitrogen ($P = 0.001$) interactions were observed for average plant height. Significantly greater plant height occurred on the High SO_2 treatment than on the Control (Figure 12.1). Plant heights for the Low and Medium SO_2 treatments were greater than the Control but were significantly lower than the High SO_2 treatment at peak plant height (∿ 5 July). Plant height was greatly enhanced by N fertilization (Figure 12.2) showing the greatest effect at peak height. The timing of peak plant height was not changed by either SO_2 or fertilizers. Significant post peak declines were evident with nitrogen fertilization.

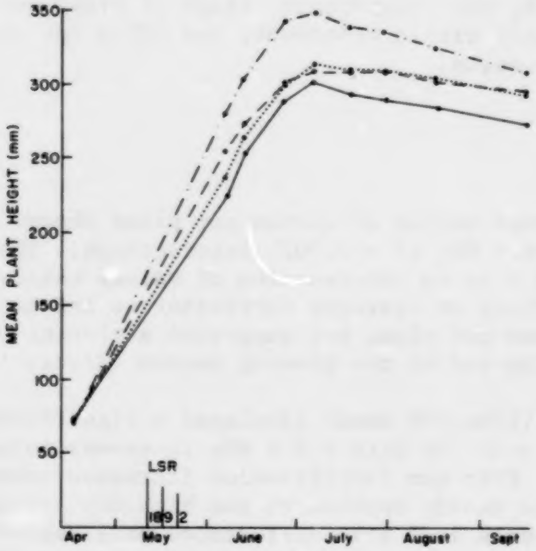

Figure 12.1. Plant height for SO_2 treatments (———— Control, — — — Low, ······· Medium, —·— High) across all fertilization treatments. Date × SO_2, $P = .055$. Use LSR_4 for significance range of SO_2 treatments within date; LSR_9 for across date within SO_2 treatment, and LSR_{18} for across SO_2 treatment across date.

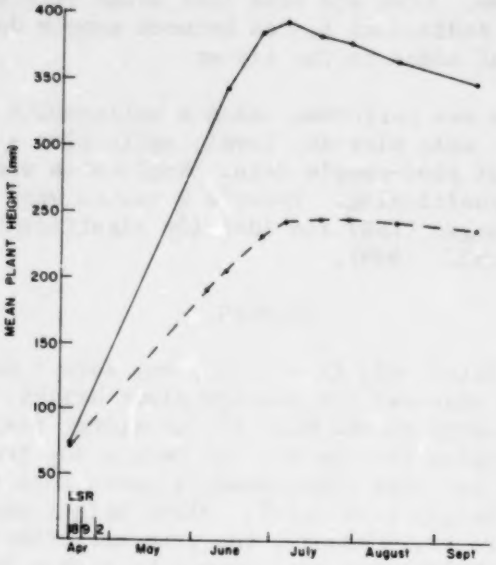

Figure 12.2. Plant height with (————) and without (— — —) nitrogen fertilization across SO_2 treatments. Date × nitrogen, P = .001. Use LSR_2 for significance range of treatments within date; LSR_9 for dates within treatment, and LSR_{18} for across treatments across dates.

Analysis of average number of leaves per plant showed significant date × N (P = 0.001) and date × SO_2 (P = 0.001) interactions. The significant time × N interaction was due to an intersection of values rather than any significant within date effect of nitrogen fertilizer as indicated by LSR values. The response to SO_2 was not clear but suggested a greater average number of leaves per plant at the end of the growing season (Figure 12.3).

Total leaf area (live and dead) displayed a significant response to N, SO_2, and sample date with the date × N × SO_2 three-way interaction significant at P = 0.021. Nitrogen fertilization increased total leaf area within all SO_2 treatments and nearly doubled on the high SO_2 treatment at peak area (Figure 12.4). Post-peak leaf area differences progressively decreased with time for SO_2 treatment with nitrogen compared to without nitrogen fertilizer. Sulfur dioxide treatment without additional nitrogen did not generally show significant differences in total standing leaf area although there was a trend toward greater leaf areas with SO_2 treatment at peak and post peak dates. Nitrogen fertilization resulted in substantial differences among SO_2 treatments. Leaf areas on the High SO_2 treatment were significantly greater than Control on all except the first and the last two dates, and were also significantly greater than those from the Low or Medium treatments up to the peak.

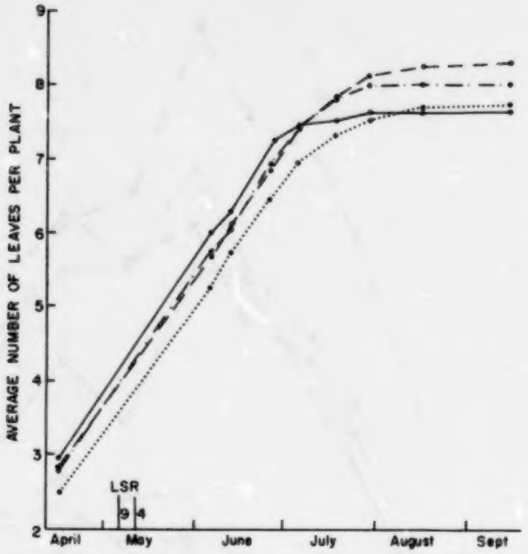

Figure 12.3. Average number of leaves per plant for SO_2 treatments. (———— Control, — — —Low, ······ Medium, —·— High) Date × SO_2, P = .001. Use LSR_4 for significance range of SO_2 treatments within date, and LSR_9 for dates within SO_2 treatment.

Post peak leaf area on the high SO_2 with nitrogen treatment declined more rapidly than other treatments and reached levels similar to the Control on the last sample date.

Analysis of total live leaf area illustrated SO_2 and nitrogen effects. Date × SO_2 and date × N interactions were both significant ($P = 0.001$). Live leaf areas of plants on the Control plot were significantly lower than those exposed to the high SO_2 concentration with the exception of the first sampling date when the plants were very small (Figure 12.5). Pre-peak increase was greater and post-peak decline was more pronounced on the High SO_2 treatment compared to Low or Medium treatments. Nitrogen fertilization produced more rapid pre-peak increases and post-peak declines in live leaf area (Figure 12.6). Very large differences between live leaf area with and without nitrogen became insignificant by the September 15 sampling date.

Total leaf area is a measure of the plant response as a whole. Examination of individual live leaf area gives a more distinct picture of the dynamics of growth because values for any given treatment/date combination do not represent an average across all leaf age classes which would include young expanding leaves as well as old senescing leaves. We will examine data

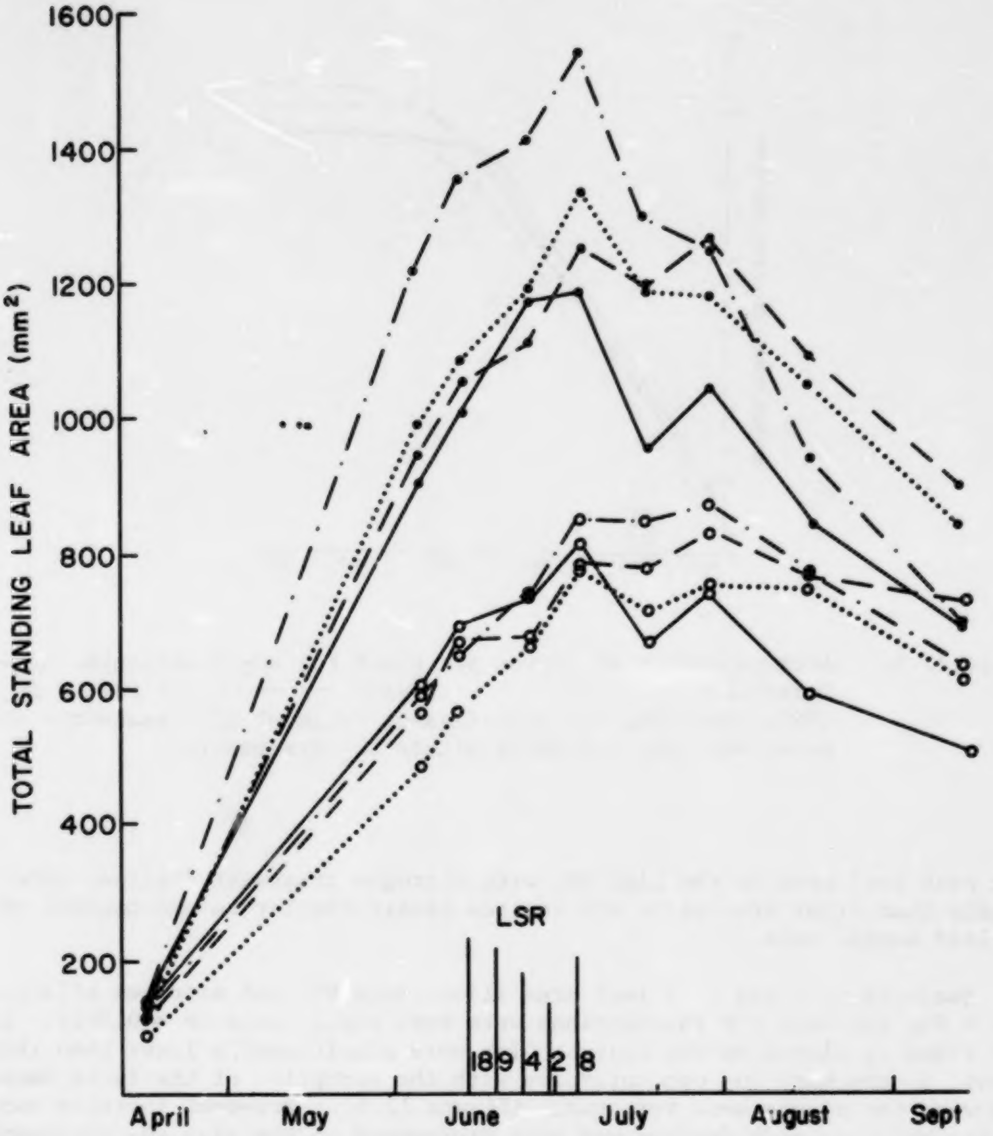

Figure 12.4. Total standing leaf area for SO_2 treatments (——— Control, — — — Low, ······ Medium, —·— High) with (●) and without (○) nitrogen fertilization. Date × N × SO_2, $P = .021$. Use LSR_2 for significance range of N fertilization within SO_2 treatment within date, LSR_4 for across SO_2 within fertilization within date, LSR_8 for across SO_2 across fertilization within date, LSR_9 for within SO_2 within fertilization across date, and LSR_{18} for across SO_2 across or within fertilization across date.

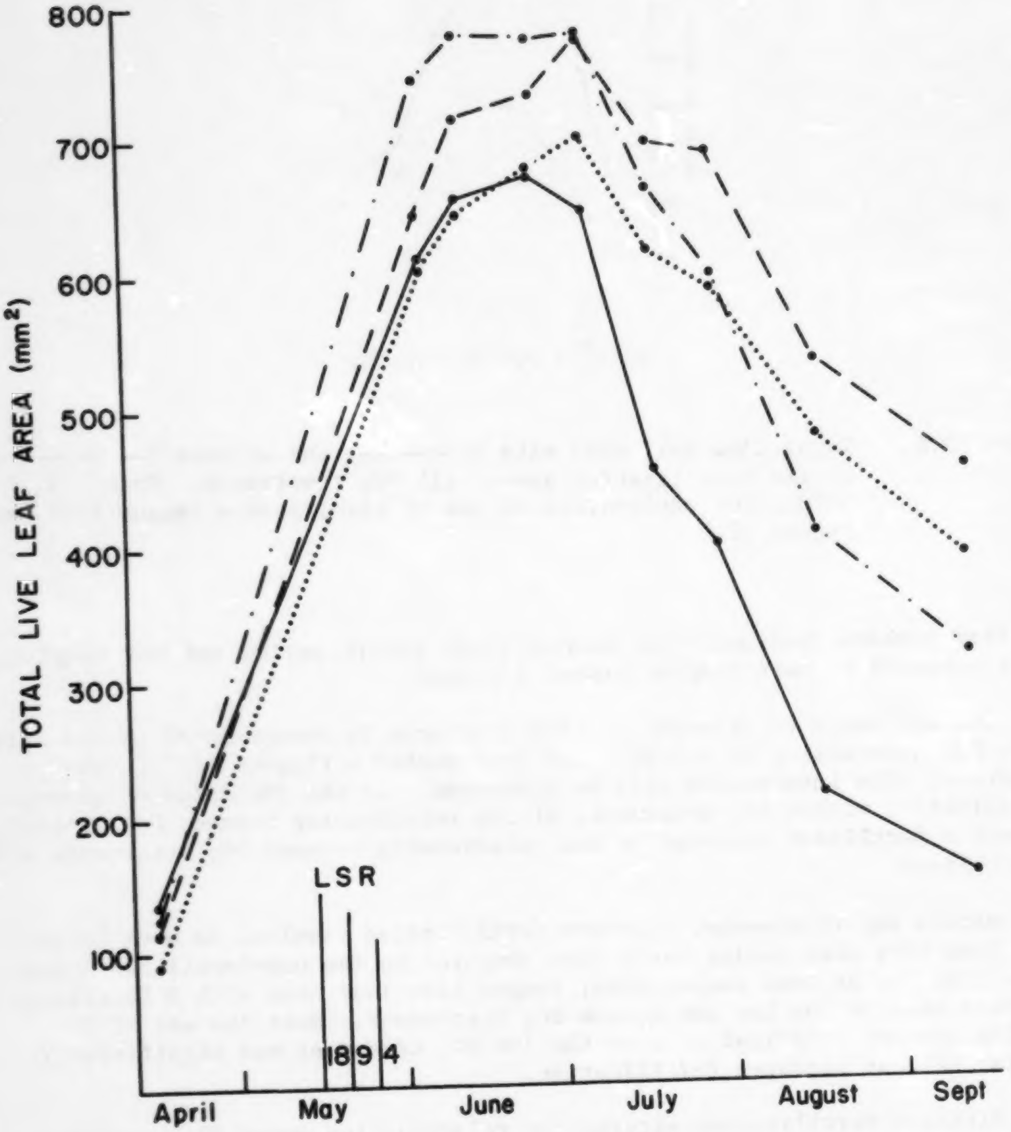

Figure 12.5. Total live leaf area for SO_2 treatments (——— Control, — — —Low, ········ Medium, —·— High) across all fertilization treatments. Date × SO_2, $P = .001$. Use LSR_4 for significance range of SO_2 treatments within date, LSR_9 for within SO_2 treatment across date, and LSR_{18} for across SO_2 treatment across date.

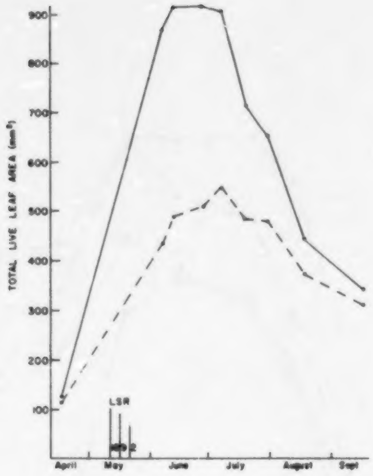

Figure 12.6. Total live leaf area with (————) and without (— — —) nitrogen fertilization across all SO_2 treatments. Date × N, P = .001. For explanation on use of significance ranges (LSR) see Figure 12.2.

for leaf numbers four and five because their growth period and our sampling dates coincide to best display growth dynamics.

The influence of nitrogen on live leaf area is demonstrated by the date × N × SO_2 interaction (P = 0.001) for leaf number 4 (Figure 12.7). Two aspects of this interaction will be discussed: 1) the influence of nitrogen fertilization within SO_2 treatment, 2) the relationship between SO_2 treatments without N fertilizer compared to the relationship between SO_2 treatments with N fertilizer.

Within SO_2 treatments, nitrogen fertilization resulted in much larger leaf four live area during early June compared to the non-fertilized treatments. By the 26 June sample date, larger live leaf area with N fertilizer was observed only on the Low and Medium SO_2 treatments. Near the end of the growing season, live leaf area on the low SO_2 treatment was significantly greater without nitrogen fertilization.

Nitrogen fertilization altered the relationships among SO_2 treatments for live area of leaf number four. Early June live leaf areas were not significantly different in the absence of N fertilizer. With N fertilizer, significant differences were observed between SO_2 treatment. A trend of distinct ordered increase in live leaf area with increasing SO_2 level was evident. By late June, exposure to SO_2 significantly increased live leaf area without nitrogen only on the Low treatment. With nitrogen fertilization, significant increases in live leaf area were observed on the Low and the Medium SO_2 treatment for June and July. Live leaf areas on all SO_2

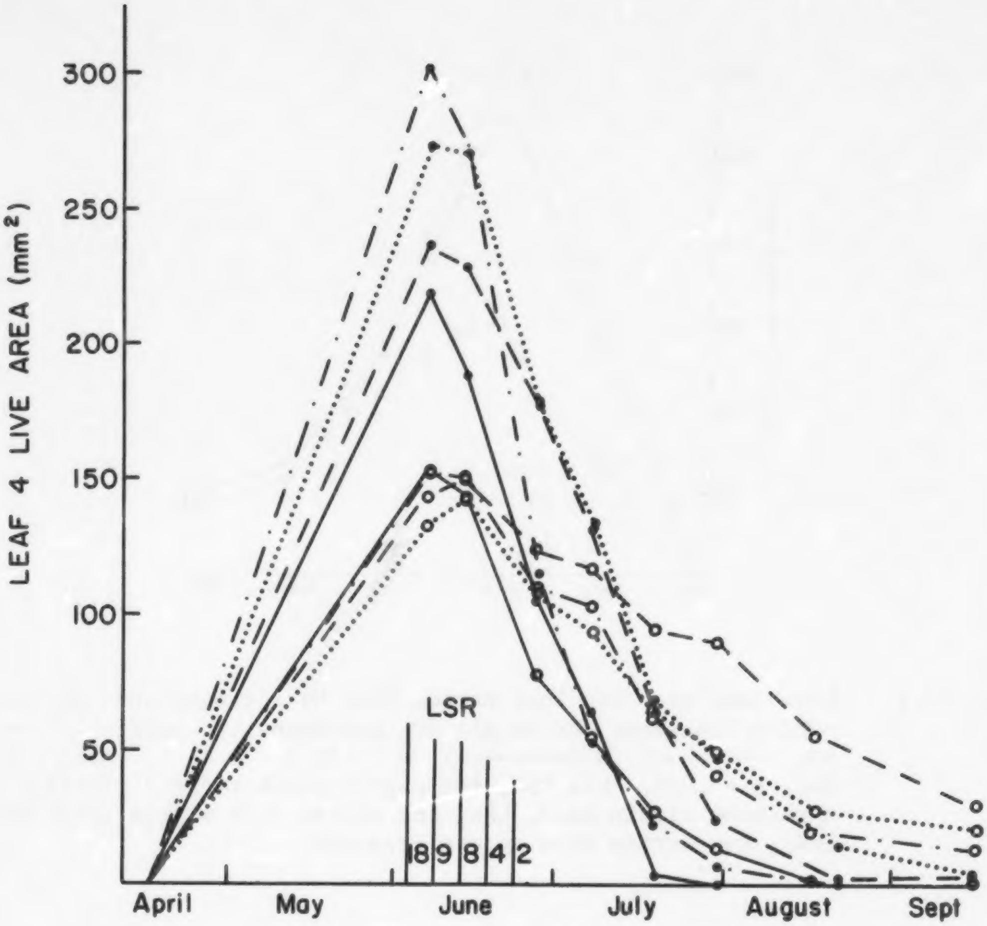

Figure 12.7. Live leaf area for leaf number four by SO_2 treatment (——— Control, — — — Low, ······· Medium, —·— High) with (●) and without (○) nitrogen fertilization. Date × N × SO_2, P = .001. For explanation on use of significance ranges (LSR) see Figure 12.4.

treatments with nitrogen converged by the 16 August sampling date. Nitrogen fertilizer induced a rapid decline in live leaf area on the high SO_2 treatment with nitrogen which was not evident on the High SO_2 treatment without nitrogen.

Sulfate fertilization had only a small influence on live leaf area as demonstrated by the significant date × N × $SO_4^=$ interaction (P = 0.036) for leaf number five (Figure 12.8). The interaction was significant largely because

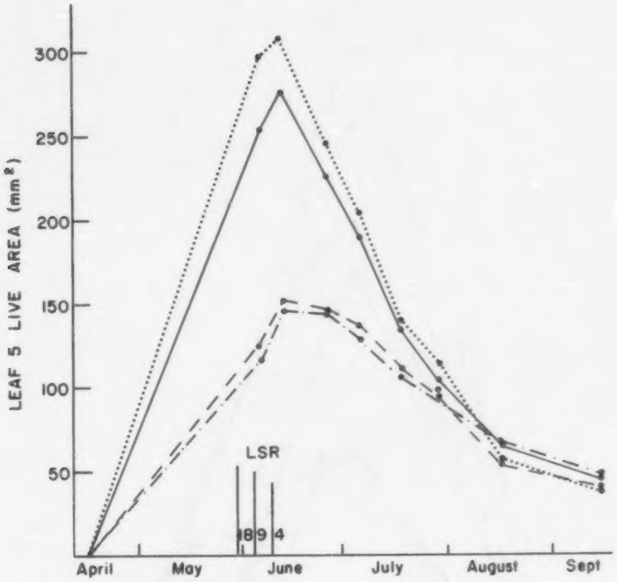

Figure 12.8. Live leaf area for leaf number five by nitrogen and sulfate fertilization treatment across all SO_2 treatments. Control (— — —), SO_4 (—— · ——), N (————), N + SO_4 (········). Date × N × SO_4, P = .036. Use LSR_4 for significance range of fertilization treatment within date, LSR_9 for across date within treatment, and LSR_{18} for across date across treatment.

of the convergence of data late in the growing season. Significant interactions involving $SO_4^=$ were not observed for any other leaf area parameter analysed. Sulfate plus nitrogen fertilizer on the non-fumigated control plants showed an increase compared to N alone but this was not statistically apparent. No sulfate × SO_2 interactions were observed.

The amount of the total calculated leaf area which was transferred to the litter during the growing season was significantly (P = 0.001) influenced by the interaction of sample date, nitrogen additions, and SO_2 exposure (Figure 12.9). Significantly greater late season litter production was observed for N compared to no N fertilization within SO_2 treatment and for the High SO_2 treatment compared to other SO_2 treatments within N fertilization.

DISCUSSION

The lack of a significant response to sulfate (without N) across SO_2 treatment is consistent with findings of Das and Runeckles (1975), Lockyer *et*

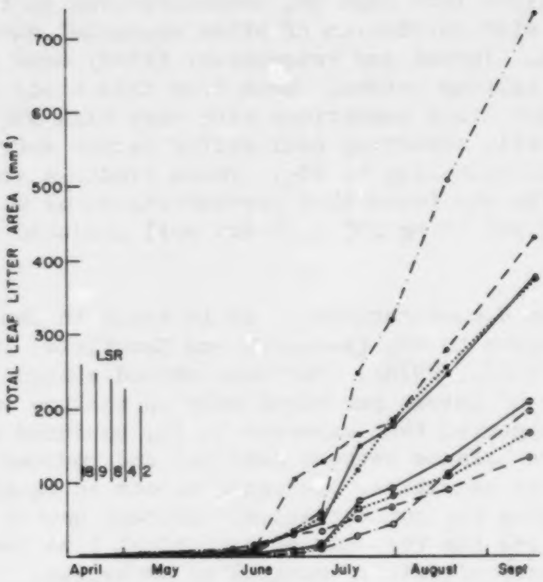

Figure 12.9. Litter area production for SO_2 treatments (——— Control, — — — Low, ········ Medium, —·— High) with (●) and without (○) nitrogen fertilization. Date × N × SO_2, P = .001. For explanation on use of significance ranges (LSR) see Figure 12.4.

al. (1976), Setterstrom *et al.* (1938), and Thomas *et al.* (1943). Faller (1971) reported minor yield variations in tobacco plants grown at 0, 40, and 80 ppm $SO_4^=$ and exposed to 57.7 pphm SO_2 but a slight yield depression with the very high 240 ppm $SO_4^=$ treatment. Eaton *et al.* (1971) reported 37 and 54 percent growth reduction in cotton and tomatoe, respectively, when 300 me/1 $SO_4^=$ was supplemented to fumigations at 7.7 pphm SO_2. Fumigating tobacco plants with 201.5 pphm SO_2, Leone and Brennan (1972) showed a positive growth response when $SO_4^=$ levels were increased from a suboptimal 1.5 ppm to 96 ppm $SO_4^=$; and then a depression in yield with 384 ppm $SO_4^=$.

Tobacco was used by both Faller (1971) and by Leone and Brennan (1972) and their 240 ppm and 384 ppm $SO_4^=$, respectively, applications were 3 and 4 times greater than the 80 ppm considered adequate for tobacco. Comparison of these studies to our $SO_4^=$ treatment is difficult because of problems in converting to ppm in field conditions. However, if we assume that $SO_4^=$ was confined to a 10 cm soil depth, soil bulk density of 1.35, and soil water of 10 percent with all $SO_4^=$ dissolved, our application of $SO_4^=$ would be 665 ppm. Regardless of the accuracy of the estimation, it is clear that $SO_4^=$ application was also very high. High soil sulfate concentrations are not toxic to plants because the tightly controlled mechanism for $SO_4^=$ uptake by roots when adequate levels are present in the plant prohibits indiscriminate uptake of additional sulfur. It is possible that high $SO_4^=$ concentrations in the soil could in some way interfere with metabolism of other essential nutrients and indirectly affect plant growth. Jordan and Reisenhauer (1957) have suggested that SO_2 may interfere with calcium uptake. Data from this study indicated no $SO_2 \times SO_4^=$ interaction under field conditions with very high $SO_4^=$ application and suggests that naturally occurring soil sulfur levels are not directly a factor in plant susceptibility to SO_2. These findings are in agreement with Lockyer *et al.* (1976) who found that concentrations of 0, 1.9, 3.8, 7.7 and 15.4 pphm SO_2 and 0 and 10 μg $SO_4^=$ kg^{-1} dry soil produced no soil treatment effect.

There have been documentations of an increase in the number of leaves per plant with exposure to SO_2 (Ashenden and Mansfield, 1977; Bleasdale, 1973; Heitschmidt *et al.*, 1978). Our data showed a significant increase in the average numbers of leaves per plant only on the Low SO_2 treatment. Bleasdale (1973) suggested that exposure to SO_2 enhanced cell division by interfering with the balance between oxidized and reduced sulfur radicals. If this mechanism was causitive, one would expect an equal or greater response with increasing SO_2 concentration. Average number of leaves per plant in this study parallel the response of individual live leaf area to SO_2 and suggests a fertilization/toxicity controlled mechanism. This is supported by reports of reduction in the number of leaves with SO_2 exposure; *i.e.*, Ashenden (1978) 11.1 pphm in *Dactylis glomerata*, Ashenden (1979) 7.5 pphm in *Poa protensis* but not *Dactylis glomerata*, Bell and Clough (1973) 13.2 pphm in S23 ryegrass.

The expected increased growth with N fertilization was observed in this study. What is interesting are comparisons of growth patterns with and without N across date, SO_2 treatment, and SO_2 treatment and date. The live leaf area of control plants with N was significantly greater at peak growth, but this did not continue as the growing season progressed. The live leaf area of plants receiving nitrogen fertilizer displayed a more rapid post peak decline and converged with the live leaf area of plants that were not fertilized with N. Further comparisons of SO_2 treatments with N to those without N show that post peak total leaf areas converged but remained higher and litter production increased at greater rates with N fertilization as the season progressed. Several important implications may be drawn from this. First it will be important to differentiate between and define rate of senescence and time of senescence. Rate of senescence is the increase in dead tissue, or the decrease in live tissue, with time. Time of senescence is the absolute amount of live tissue remaining at a particular time. In comparing between two treatments then, rate of senescence may be greater even though time of senscence is not. This was the pattern observed in plants fertilized with nitrogen compared to plants not receiving nitrogen fertilizer. Rate of senescence can be a function of a fertilization effect rather than a toxic effect and has no bearing on toxicity unless absolute live areas are also reduced. The large differences in treatments observed at the height of the growing season with the eventual converging of data as the growing season progressed also indicates that final harvest data may underestimate treatment effects when consumption occurs throughout the season as in a grazing rather than a crop situation.

Without N fertilization significantly increased plant growth was observed only on the Low SO_2 treatment. Live leaf areas for the Medium and High SO_2 treatments were neither significantly lower than the Low SO_2 nor significantly higher than the Control. This may be viewed as a peak SO_2 fertilization effect at the Low concentration and approaching the point on the High SO_2 treatment where toxic effects begin to counteract the fertilization effect.

With N fertilization a quite different response was observed between SO_2 treatments. Nitrogen fertilization resulted in increased peak growth with increasing level of SO_2. Post-peak growth was significantly greater on Low and Medium SO_2 treatments with N, while only significantly greater on the Low SO_2 treatment without N fertilization. Our highest SO_2 concentration with N fertilization displayed a time shift in leaf area distribution. Total leaf area was greater than all other treatments at pre-peak and peak growth periods and then declined more rapidly. Post peak live leaf area on the N plus High SO_2 treatment was also significantly less than those found for the Low or Medium SO_2 treatments. Litter area within N fertilization was significantly greater for the High SO_2 treatment. These factors indicate a more rapid rate of senescence and time of senescence for High SO_2 compared to Low or Medium SO_2 with N fertilization, but only a more rapid rate of senescence compared to the Control with N fertilization. Because of the increased growth associated with SO_2 plus N fertilization, the observed senescence pattern may be the result of a shift in phenology with earlier development and senescence, as was observed with N fertilization alone, rather than a toxic effect which would depress live leaf areas and growth. Differentiating between rate and time of senescence is necessary when examining the effects of any compound which can be both a fertilizer and a toxic substance.

Cotrufo and Berry (1970), Scurfield (1969), Zahn (1963), Enderlein and Kästner (1967), Krauss (1967) and Materna and Kohout (1967) have also observed higher resistance to SO_2 in fertilized plants. In Cotrufo and Berry's study, 0.5 g of NPK fertilizer per pot sharply decreased pine needle injury from SO_2. With 1 g of fertilizer per pot, even less injury was observed. However, when 2 g of fertilizer per pot was applied, tip necrosis was again evident. Cotrufo and Berry could not explain a cause for the increased sensitivity to SO_2 with high fertilizer applications but suggested there was an interaction between high salt concentration of the needles and air pollution. In this study, N application was constant across three concentrations of SO_2. We also observed an ameliorating effect of nitrogen fertilization on SO_2 exposure with an increased rate and time of senescence on the high SO_2 with N treatment. These data indicate that the $SO_2 \times N$ interaction can be both positive and negative and that the threshold between positive and negative can be altered by varying either fertilizer or SO_2 concentrations. Our data further indicate that the assessment of positive or negative effects of any one combination of SO_2 and N is dependent on time. A positive high $SO_2 \times N$ effect was observed during the peak growth period while negative effects were observed during the post-peak growth period.

Studies on the effect of SO_2 on plant nitrogen metabolism suggest a possible explanation for the increased rate of senescence on the High SO_2 plus N fertilized plants and the observation of Cotrufo and Berry (1970). Changes in free amino acid concentrations and enzymes involved in amino acid metabolism have been found to be a typical response to SO_2 (Jäger, 1975; Jäger and Klein, 1977; Wellburn *et al.*, 1976; Godzik and Linskens, 1974; Malhotra and Sarkar, 1979). Increases in free amino acid content at higher SO_2 concentrations are probably brought about by protein hydrolysis (Malhotra and Sarkar, 1979). Protein hydrolysis can eventually lead to tissue senescence (Fischer, 1971). Steinberg *et al.* (1950) proposed that the chlorosis commonly shown by plants subjected to nutritional stress is the result of an accumulation of free amino acids. High NH_3 levels are also observed in plants exposed to SO_2 (Godzik and Linskens, 1974; Jäger and Klein, 1977). Both SO_2 and ammonium nutrition increase the H^+ ion concentration of cells and cause a shift in the cation/anion ratio. When the buffering capacity of the tissues is exhausted, cell pH decreases. Sulfur dioxide and N, while exhibiting a synergistic fertilizer effect at low concentration, may in combination at higher concentrations result in detrimental effects.

The significant response to N fertilization within and between SO_2 treatments observed in this study demonstrates that N can ameliorate the effects of SO_2 pollution by increasing the amount of sulfur plants can use for growth and metabolism and thereby increase the atmospheric toxic concentration threshold. However, negative effects may also result from nitrogen fertilization with SO_2 exposure. The balance between negative or positive effects on plant growth is dependent on a complex interaction between soil nitrogen level, SO_2 concentration, and time. The validity of generalized toxic concentration levels must be questioned when one considers that in addition to soil nutrient status the effect of a given SO_2 concentration can change in response to interactions of soil moisture, humidity, temperature, wind, light, diurnal SO_2 fluctuation, synergism with other air pollutants, and plant species composition.

In a natural rangeland system the impact of fertilization or SO_2 pollution must be assessed not only in terms of total productivity but also in view of changes in the distribution of the resource in time and the quality of the resource to consumers. Forage quality of western wheatgrass from this study area has previously been examined (Milchunas *et al.*, 1980). The distribution in time effects observed in this study could have important implications especially for non-domestic consumers. A more rapid rate of senescence may affect nutrient density by altering live to dead ratios and thereby affect consumers. Rumen bulk capacity and the rate of digesta breakdown and passage through the alimentary tract can limit forage intake before the animals' requirements for energy or nutrients are met (Milchunas *et al.*, 1978). Earlier senescence, irrespective of peak and mid-summer production, can affect the length of time wild ruminants are subject to low quality forage. This is a major factor in winter survival and spring natality rates.

CONCLUSIONS

No interaction of soil sulfate with SO_2 concentration was observed even though SO_4 fertilizer applications were high. Average number of leaves per plant paralleled live leaf area responses to SO_2 and suggests a fertilization/toxicity controlled mechanism rather than SO_2 enhanced cell division. Sulfur dioxide exposures significantly increased leaf area only on the low SO_2 treatment. Nitrogen fertilization can ameliorate the effects of SO_2 but can also cause increased rate and time of senescence at higher SO_2 concentrations. Nitrogen fertilization in the absence of SO_2 can result in a more rapid rate of senescence. Differentiating between rate and time of senescence is necessary when examining the effects of any compound which can both be a fertilizer and a toxic substance. Senescence can have an effect on consumers by altering the seasonal resource distribution.

REFERENCES

Ashenden, T. W. 1978. Growth Reductions in Cocksfoot (*Dactylis glomerata* L.) as a Result of SO_2 Pollution. Environ. Pollut., 15:161-166.

Ashenden, T. W. 1979. The Effects of Long-term Exposures to SO_2 and NO_2 Pollution on the Growth of *Dactylis glomerata* L. and *Poa pratensis* L. Environ. Pollut., 18:249-257.

Ashenden, T. W., and T. A. Mansfield. 1977. Influence of Wind Speed on the Sensitivity of Ryegrass to SO_2. J. Exp. Bot., 28:729-735.

Aulakh, M. S., G. Dev, and B. R. Arora. 1976. Effect of Sulphur Fertilization on the Nitrogen-sulfur Relationships in Alfalfa (*Medicago sativa* L. Pers.). Plant and Soil, 45:75-80.

Bell, J. N. B., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulfur Dioxide. Nature, 241(5384):47-49.

Bleasdale, J. K. A. 1973. Effects of Coal-smoke Pollution Gasses on the Growth of Ryegrass (*Lolium perenne* L.). Environ. Pollut., 5:275-285.

Booth, J. A., G. O. Thorneberry, and M. Lujan. 1976. Crop Reactions to Sulfur Dioxide in New Mexico. New Mexico State University Ag. Exp. Sta. Bulletin 645. 27 p.

Brisley, H. R., and W. W. Jones. 1950. Sulfur Dioxide Fumigation of Wheat with Special Reference to its Affect on Yield. Plant Physiol., 25:666-681.

Brisley, H. R., C. R. Davis, and J. A. Booth. 1959. Sulfur Dioxide Fumigation of Cotton with Special Reference to its Effect on Yield. Agron. J., 51:77-80.

Constantinidou, H. A., and T. T. Kozlowski. 1979. Effects of Sulfur Dioxide and Ozone on *Ulmus americana* Seedlings. II. Carbohydrates, Proteins, and Lipids. Can. J. Bot., 57:176-184.

Cotrufo, C., and C. R. Berry. 1970. Some Effects of a Soluble NPK Fertilizer on Sensitivity of Eastern White Pine to Injury from SO_2 Air Pollution. Forest Sci., 16:72-73.

Cowling, D. W., and D. R. Lockyer. 1978. Effect of SO_2 on *Lolium perenne* Grown at Different Levels of Sulfur and Nitrogen Nutrition. J. Exp. Bot., 29:257-166.

Daines, R. H. 1968. Sulfur Dioxide and Plant Response. J. Occupational Med., 10:516-524.

Das, G., and V. C. Runeckles. 1975. Bisulphite-induced Inactivation of Growth and Chlorophyll Formation in *Chlorella pyrenoidosa*. J. Exp. Bot., 26:705-712.

Dijkshoorn, W., J. E. M. Lampe, and P. F. J. Van Burg. 1960. A Method of Diagnosing the Sulphur Nutrition Status of Herbage. Plant and Soil, 13:227-241.

Eaton, F. M., W. R. Almstead, and O. C. Taylor. 1971. Salt Injury to Plants with Special Reference to Cations Versus Anions and Ion Activities. Plant and Soil, 35:533-547.

Enderlein, H., and W. Kästner. 1967. Welchen Einfluss hat der Mangel eines Nährstoffes auf die SO_2-Resistenz einjähriger Kiefern. Arch. Forstw., 16: 431-435.

Faller, N. 1971. Plant Nutrient Sulphur - SO_2 vs. SO_4. Sulfur Inst. J., 7:5-6.

Fischer, K. 1971. Methoden zur Erkennung und Beurteilung forstschadlicher Luftverunreinigungen. Chemische und physikalische Reaktionen SO_2-begaster Pflazen und Blatter. Mitt. Forstl. Bundes-Versuchsanst. Wain, 92:209-231.

Garsed, S. G., and D. J. Read. 1977. The Uptake and Metabolism of $^{35}SO_2$ in Plants of Differing Sensitivity to Sulphur Dioxide. Environ. Pollut., 13:173-186.

Godzik, S., and H. F. Linskens. 1974. Concentration Changes of Free Amino Acids in Primary Bean Leaves After Continuous and Interrupted SO_2 Fumigation and Recovery. Environ. Pollut., 7:25-38.

Guderian, R. 1977. Air Pollution. Ecological Studies 22. Springer-Verlag, Berlin. 118 p.

Guderian, R., and H. Stratmann. 1962. Freilandversuche zur Ermittlung von Schwefeldorydwirkungen auf die vegetation. Forschungberichte des landes Nordrhein-Westfallen, 1118:7-102.

Guderian, R., and H. Van Haut. 1970. Detection of SO_2-Effects Upon Plants. Staub, 30:22-35.

Heitschmidt, R. K., W. K. Lauenroth, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulphur Dioxide on Western Wheatgrass in a Southeastern Montana Grassland. J. Appl. Ecol., 14:859-868.

Jäger, H. J. 1975. Zur saulenchrumatographichen Bestimmung freier Aminosauren and Diamine in Pflanzenmaterial. Angew. Bot., 49:25.

Jäger, H. J., and H. Klein. 1977. Biochemical and Physiological Detection of Sulfur Dioxide Injury to Pea Plants (*Pisun satirun*). APCA J., 27:464-465.

Jordan, H. V., and H. M. Reisenhauer. 1957. Sulfur and Soil Fertility. In: USDA Yearbook of Agriculture, Soils. p. 107-111.

Katz, M. 1949. Sulfur Dioxide in the Atmosphere and its Relation to Plant Life. Ind. Eng. Chem., 41:2450-2465.

Krauss, H. H. 1967. Dungungsversuche an ranchgesschadigten Kiefernbestanden der Dubener Heide. Arch. Forstw., 15:1145-1163.

Leone, I. A., and E. Brennan. 1972. Sulfur Nutrition as it Contributes to the Susceptibility of Tobacco and Tomato to SO_2 Injury. Atmos. Env., 6:259-266.

Lockyer, D. R., D. W. Cowling, and L. H. P. Jones. 1976. A System for Exposing Plants to Atmospheres Containing Low Concentrations of Sulphur Dioxide. J. Exp. Bot., 27:397-409.

Malhotra, S. S., and D. Hocking. 1976. Biochemical and Cytological Effects of Sulphur Dioxide on Plant Metabolism. New Phytol., 76:227-237.

Malhotra, S. S., and S. K. Sarkar. 1979. Effects of Sulphur Dioxide on Sugar and Free Amino Acid Content of Pine Seedlings. Physiol. Plant., 47:223-228.

Materna, J. and R. Kohout. 1967. Stickstoff-Dungung and Schwefeldioxyd-Aufnahme durch Fichtennadeln. Naturwissenschaften, 54:351.

Medvedev, Z. A. 1957. Content of Methionine, Cystine and Total Sulfur in Proteins of Various Intracellular Fractions of Kidney-bean and Sunflower Leaves as a Function of the Age of the Plants. Biokhimiya, 22:855-864 (translation - Biochem., 22:806-815).

Milchunas, D. G., M. I. Dyer, O. C. Wallmo, and D. E. Johnson. 1978. *In vivo/in vitro* Relationships of Colorado Mule Deer Forages. Colorado Division of Wildlife Special Report Number 43. 44 p.

Milchunas, D. G., W. K. Lauenroth, and J. L. Dodd. 1980. Forage Quality of *Agropyron smithii* Exposed to Sulfur Dioxide. In: Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report , Colstrip, Montana. E. M. Preston and D. W. O'Guinn (eds.) EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 185-196.

Pumphrey, F. V., and D. P. Moore. 1965. Sulphur and Nitrogen Content of Alfalfa Herbage During Growth. Agron. J., 57:237-239.

Rendig, V. V., and E. A. McComb. 1959. Effect of Nutritional Stress on Plant composition: I. The Interaction of Added Nitrogen with Varying Sulfur Supply. Soil Sci. Soc. Proc., 23:377-380.

Salesbury, F. B., and C. Ross. 1969. Metabolism and Functions of Nitrogen and Sulfur. In: Plant Physiology. Wadsworth Publ. Co., Calif. 747 p.

Saunders, P. J. W., and C. M. Wood. 1973. SO_2 in the Environment, its Production, Dispersal and Fate. In: Air Pollution and Lichens, B. W. Ferry, M. S. Baddeley and D. L. Hanksworth, eds., Anthlone Press, London.

Scurfield, G. 1969. Air Pollution and Tree Growth. Forest Abstr., 21:339-347, 517-528.

Setterstrom, C., P. W. Zimmerman, and W. Crocker. 1938. Effect of Low Concentrations of Sulfur Dioxide on Yield of Alfalfa and Cruciferae. Contrib. Boyce Thompson Inst. Plant Res., 9:179-198.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. Wit Freeman and Co., San Francisco. 776 p.

Steinberg, R. A., J. D. Bowling, and J. E. McMurtrey, Jr. 1950. Accumulation of Free Amino Acids as a Chemical Basis for Morphological Symptoms in Tobacco Manifesting Drenching and Mineral Deficiency Symptoms. Plant Physiol., 25:279-288.

Stern, A. 1968. Air Pollution. Academic Press, NY.

Thomas, M. D., R. H. Hendricks, L. C. Bryner, and G. R. Hill. 1944. A Study of the Sulfur Metabolism of Wheat, Barley and Corn Using Radioactive Sulfur. Plant Physiol., 19:227-244.

Thomas, M. D., R. H. Hendricks, T. R. Collier, and G. R. Hill. 1943. The Utilization of Sulphate and Sulphur Dioxide for the Sulphur Nutrition of Alfalfa. Plant Physiol., 18:345-371.

Tingey, D. T., W. W. Heck, and R. A. Reinert. 1971. Effect of Low Concentrations of Ozone and Sulfur Dioxide on Foliage, Growth and Yield of Radish. J. Amer. Soc. Hort. Sci., 96:369-371.

Wellburn, A. R., T. M. Capron, H. S. Chan, and D. C. Horsman. 1976. Biochemical Effects of Atmospheric Pollutants on Plants. In: Effects of Air Pollutants on Plants, T. A. Mansfield, ed. Cambridge University Press. p. 105-114.

Zahn, R. 1963. Über den Einfluss verschiedener Umweltfaktoren auf die Planzenempfindlichkeit gegennüber Schwefeldiaxyd. Z. Pflkrankh. Pfl. Path., Pflschutz, 70:81-95.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79-105.

EFFECTS OF CHRONIC SO_2 EXPOSURE ON POPULATION AND COMMUNITY STRUCTURE

SECTION 13

SEED GERMINATION AND SEEDLING ESTABLISHMENT AS AFFECTED BY SULFUR DIOXIDE

W. C. Leininger and J. E. Taylor

ABSTRACT

The effects of sulfur dioxide fumigation on range soils, seed germination, and seedling establishment were studied in the field and in the greenhouse. Unpolluted soil samples were seeded with seven perennial indigenous plant species and exposed to four levels of SO_2 fumigation in the field. Soils subjected to five growing seasons of fumigation were seeded similarly and placed in an unpolluted greenhouse. In the field, soil sulfur levels after 8 weeks fumigation increased significantly with rate. Green needlegrass (*Stipa viridula*) exhibited leaf necrosis on the highest sulfur treatment. Tissue sulfur of all species increased with fumigation intensity. In the greenhouse, the soils from the highest sulfur plot (D) had 6 times the sulfur content of the control (A). Germination and establishment were significantly reduced for five species. Nongraminoids were particularly affected. Bluebunch wheatgrass (*Agropyron spicatum*) and green needlegrass accumulated significantly more sulfur on D-plot than on A-plot soils. Plant height and leaf number were not significantly affected at any fumigation level. Volunteer species were more abundant and diverse on the two lowest sulfur soils.

INTRODUCTION

Species composition of plant communities affected by chronic air pollution may change as species differentially respond to the stress factors present. Given adequate time, species may undergo genetic adaptation. More immediate symptoms could be loss of sensitive species and/or pathological signs in others. The following changes have been observed in plants in response to SO_2 stress (ZAPS I and II): increased leaf senescence in western wheatgrass (Dodd *et al.*, 1979a); reduced plant canopy coverage and species diversity and species composition changes (Taylor and Leininger, 1979; Taylor *et al.*, 1980); and reduced reproductive viability (Rice *et al.*, 1980).

Species which do not show strong fluctuations in population density may exhibit an evolutionary tendency toward lower sexual reproduction (Stearns, 1977). In many temperate rangeland plant species this occurs, with vegetative propagation assuming primary significance in the perpetuation and replacement of most perennial species. Nevertheless, when disturbances occur which change one or more of the habitat's environmental variables to levels beyond the tolerance or competitive adaptability of some species, sexual reproduction is necessary for replacement by other species. Also, upon the subsequent relief from such disturbances, former site occupants which have not survived the stress period must re-enter the system by sexual means, if at all. In such situations, the reproductive performances of the component plant species are critical to the community's ability to adapt (Harper, 1965).

Seed development and dispersal and seedling establishment are complex facets of sexual reproduction. Rice *et al.* (1980) looked at seed development; we studied seed germination and seedling establishment under SO_2 stress. The study occurred in two phases: field observations of seedling establishment under SO_2 fumigation on soils which had not been fumigated previously, and greenhouse observations of seeds germinated in "clean" air in soils which had been fumigated for five growing seasons.

Lauenroth *et al.* (1980) stated that sulfur concentrations within plants were a function of growth rate, duration of growing conditions, concentration of SO_2, duration of exposure to SO_2, and concentration of SO_2 in the soil sulfur pool. In order to isolate soil sulfur effects, we examined sulfur accumulation in the tissues of bluebunch wheatgrass (*Agropyron spicatum*) and green needlegrass (*Stipa viridula*), the only two of seven species producing enough plant tissue for the analysis.

MATERIALS AND METHODS

Field Studies

Samples of soil were removed from the A-horizon (approximately top 7 cm) of an area west of the ZAPS I site; slope, orientation, elevation, and vegetation of the collection site were similar to ZAPS I. The collection site had not been subject to sulfur fumigation due to distance and direction from the fumigation site. Soil was placed in perforated metal trays 34 x 50 x 9 cm deep. One row of each of seven plant species (Table 13.1) was planted at a relatively high density per tray. All seeds were from indigenous local collections on nonfumigated sites. Species were selected because of their economic importance and abundance in southeastern Montana.

Seeding rates on a pure live seed (PLS) basis were not calculated because the purpose of the study was to observe establishment, sulfur accumulation and pathologic signs rather than percent germination. It was assumed that since both soils and seeds had been taken from unpolluted sites, seed germination

TABLE 13.1. NATIVE PERENNIAL PLANT SPECIES USED IN GERMINATION/ESTABLISHMENT STUDIES

Common Name	Scientific Name	Season of Growth	Plant Type
Western wheatgrass	*Agropyron smithii*	Cool	Grass
Bluebunch wheatgrass	*Agropyron spicatum*	Cool	Grass
Sideoats grama	*Bouteloua curtipendula*	Warm	Grass
Green needlegrass	*Stipa viridula*	Cool	Grass
Wild flax	*Linum perenne*	Cool	Forb
Purple prairieclover	*Petalostemon purpureum*	Warm	Forb
Fourwing saltbush	*Atriplex canescens*	Warm	Shrub

should not be affected by sulfur fumigation in the time period when the pans were exposed. The species were randomly assigned to rows within each pan. Four replicated trays were prepared for each fumigation treatment and an additional tray was planted and taken to Bozeman to monitor plant development. After planting (22 May), each tray was watered to field capacity and then a set of four replicates placed on each of the ZAPS treatment plots, in locations geometrically similar to the locations of gas monitor C in each treatment.

Trays were watered during the study to maintain good growing conditions. On 20 June and 28 June (29 and 37 days from date of planting) trays were photographed and examined for seedling number, average seedling height, and average number of leaves for each species. A record of leaf senescence also was obtained on 28 June.

On 25 July (64 days after planting) trays were taken to the laboratory for analyses. Average plant height and average number of leaves per species were calculated. Average length of longest leaf for each individual was determined and length of necrotic tissue of the longest leaf measured. All plants were clipped at ground level and roots were exhumed for sulfur analysis.

Aboveground plant tissue was analyzed for sulfur using the procedures of Tabatabai and Bremner (1970) as employed by Hanson (1976); soil samples were analyzed with techniques of Bardsley and Lancaster (1960). For no species was there sufficient root biomass for analysis.

Greenhouse Studies

Soils were collected from ZAPS I plots A-D on 17 October 1979. Collections were within 4 cm of the soil surface (area of greatest natural seed germination) and at the same distance from the gas exit pipes as monitoring sensor C. Soils were analyzed for total sulfur using techniques of Bardsley and Lancaster (1960).

Hand sorted, filled seeds of western wheatgrass, bluebunch wheatgrass, and green needlegrass (100 seeds of each), and sideoats grama, purple prairie-clover, wild flax, and fourwing saltbush (50 seeds of each) were planted in randomized rows in trays. There were four replicate trays per treatment. Trays were placed under Sylvania Grow-Lux lights, periodically watered, and allowed 12 hours of light per day to provide favorable growing conditions.

Number of seedlings, average number of leaves, and height of each species were recorded every second or third day for 38 days (through 3 March 1980). Maximum germination was reached by day 17 for six species; western wheatgrass reached maximum germination on day 25. Plants were allowed to grow to accumulate enough tissue biomass to be analyzed.

At the end of the experiment plants were harvested. Bluebunch wheatgrass and green needlegrass, grown in the A and D treatment soils, were analyzed for sulfur accumulations.

During the experiment, a striking difference in the numbers of volunteer species (germinating from residual seeds in soil) was noted. After an additional three weeks of growth, these plants were identified to species and counted in each tray; species diversity (H') (Shannon and Weaver, 1949) and species richness (number of species) were calculated for each treatment.

RESULTS AND DISCUSSION

Field Studies

The sulfur content of the soils increased with fumigation (Figure 13.1). This increase was linear ($P \leq 0.01$) with exposure. The fumigation rates used in the correlations were monthly averages weighted by the number of days of exposure in each month of the study. Sulfur dioxide values were A = 1.23; B = 2.46; C = 4.43; and D = 6.85 pphm. The soil from the A plot had a significantly higher sulfur level than the sample which had been removed to Bozeman, suggesting contamination on the ZAPS Control. For this analysis, the Bozeman site was assumed to be a sulfur-free environment, although no monitoring data were available. The actual site was approximately 6 km south of Bozeman in a suburban setting with minimal sulfur sources nearby or upwind.

The variation associated with sulfur measurements was quite low so that statistically significant step differences were detected between ZAPS A and between levels. The B and C exposure plots did not differ ($P \leq 0.05$).

The phenomenon of sulfur accumulation in soils is well documented in the literature (Eaton and Eaton, 1926; Wilson, 1921). Bertramun *et al.* (1949) documented absorption of atmospheric sulfur by both soils and plants, using isotopic tracer techniques. In the vicinity of a pollution source, the actual amount of sulfur absorption by soils is a function of distance and soil texture (McCoal and Mehlich, 1938). They noted that silty soils showed a differential sulfur accumulation with distance from pollution sources, but some clay soils did not. In our study using silty clay loam soils (Dodd *et al.*, 1979b), accumulation occurred over a very short time, about 8 weeks.

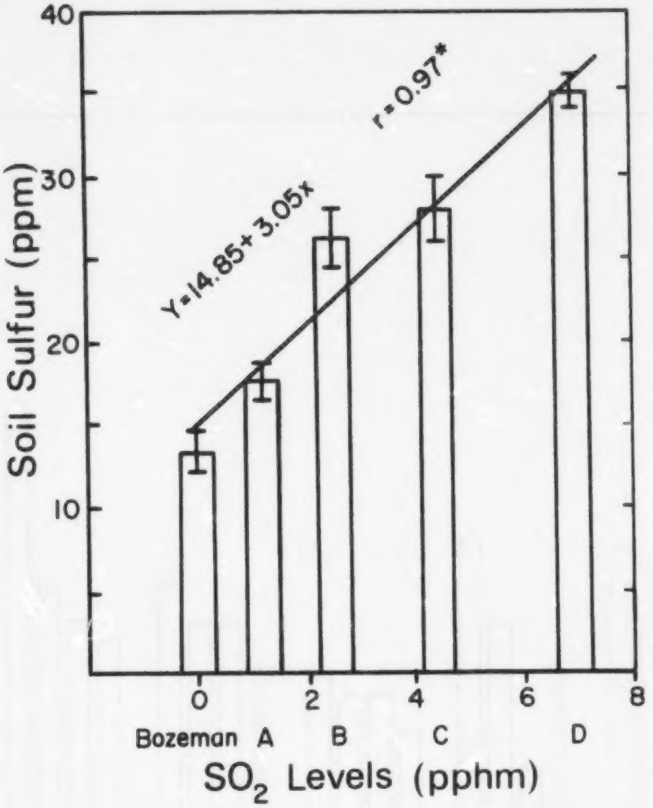

Figure 13.1. Soil sulfur accumulated on ZAPS I and Bozeman control after 64 days of fumigation (22 May - 25 July, 1979). Mean level ± 1 standard error is shown; *P ≤ 0.01.

Plants in the field were seriously damaged by rodent and rabbit depredations on the growing seedlings. Repeated defoliation reduced total plant material for sulfur analysis and also limited our ability to take adequate measurements of leaf numbers and lengths and plant numbers.

Green needlegrass showed conspicuous leaf necrosis (up to one-fourth of the leaf length on D treatment) with SO_2 exposure. The damaged was similar to that described by Treshow (1970) and not observed on other species.

The accumulation of sulfur in aboveground plant tissue under SO_2 fumigation is shown in Figure 13.2. Wild flax was not analyzed because it lacked adequate material. Tissue sulfur levels in western wheatgrass were similar to those reported by Dodd *et al.* (1979a) and Rice *et al.* (1979). Green needlegrass values approximated those of Rice *et al.* (1980). These were the only species in common with their studies and ours. Powers (ND) found that tissue sulfur was higher in actively growing plants. The similarity between

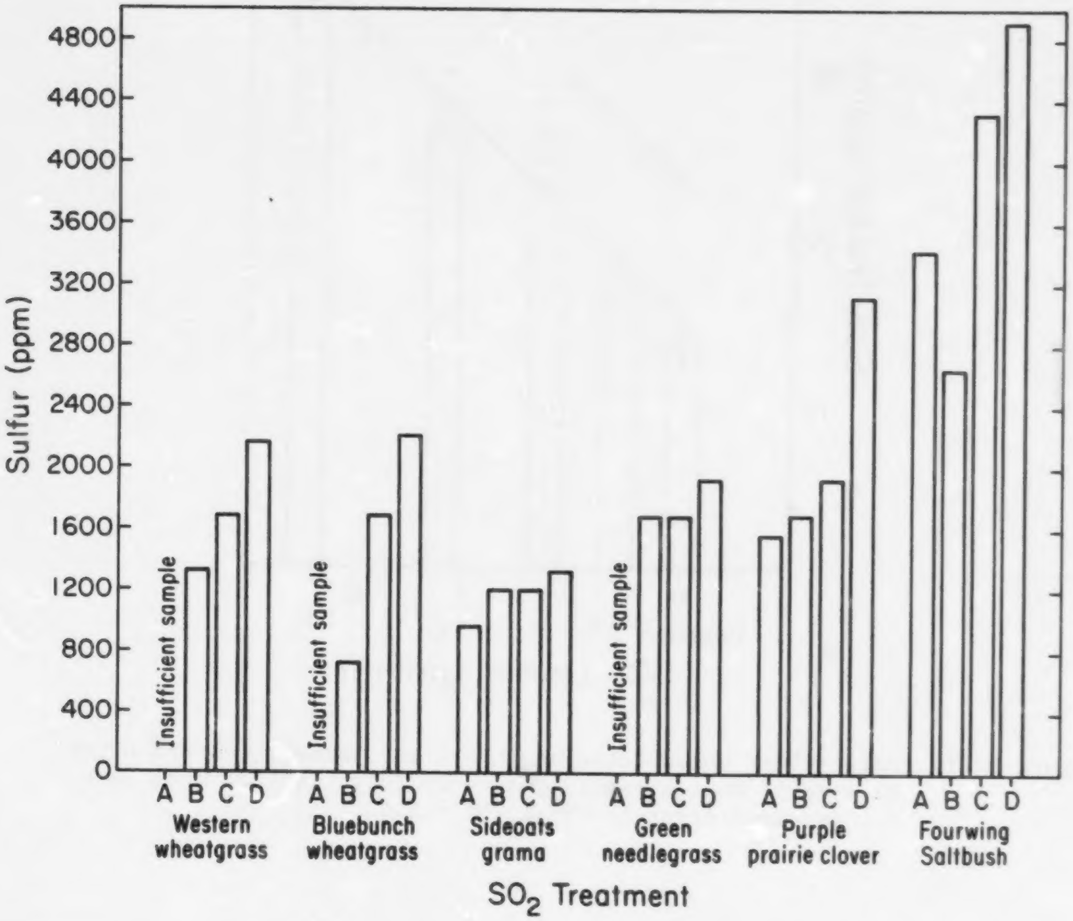

Figure 13.2. Accumulation of sulfur in aboveground plant tissue under SO_2 fumigation.

our observations in seedlings and those compared for mature species suggest that growth stage makes little difference as long as plants are actively metabolizing. There was insufficient root biomass of any species for chemical analysis.

Plant sulfur correlations are presented in Table 13. 2. All species showed positive correlations between sulfur fumigation and plant sulfur content.

TABLE 13.2. LINEAR REGRESSION AND CORRELATIONS BETWEEN PLANT SULFUR AND SO_2 FUMIGATION, ZAPS I, 1979

Species	Regression Equation	r	r^2
Western wheatgrass	$Y = 842.4 + 101.6\ x$	1.00*	1.00
Bluebunch wheatgrass	$Y = 4.5 + 337.2\ x$	0.98	0.95
Sideoats grama	$Y = 968.0 + 53.96\ x$	0.88	0.77
Green needlegrass	$Y = 1501.9 + 56.3\ x$	0.89	0.80
Purple prairieclover	$Y = 1047.5 + 273.2\ x$	0.94	0.88
Fourwing saltbush	$Y = 2525.0 + 347.2\ x$	0.85	0.72

* $P \le 0.05$.

Although Treshow (1970) pointed out the problem of quantifying atmospheric sulfur levels from plant sulfur contents and/or the degree of plant injury, our data suggest that within a local vegetation type and climatic regime such quantification may be feasible.

Greenhouse Studies

There were significant increases in sulfur among all treatments, with the major increase between treatments C and D (Figure 13.3). The mean sulfur level for treatment D was more than six times the level for treatment A.

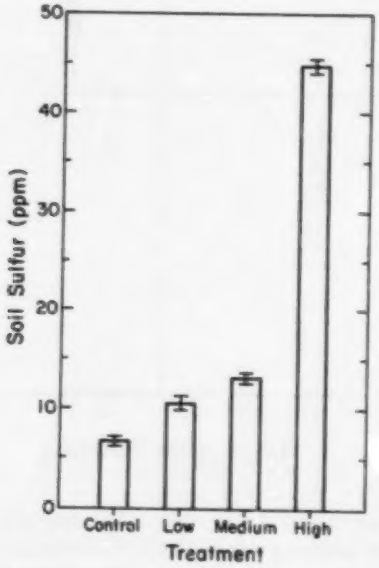

Figure 13.3. Soil sulfur accumulation after five growing seasons of fumigation. Mean ± 1 standard error is shown.

Differences may have indicated resistance to removal of sulfur at the lower three levels of fumigation and/or a threshold fumigation rate (somewhere between C and D) against which plants do not effectively remove sulfur. It also could show the secondary effects of plant growth inhibition at higher fumigation levels. Because of the reduced plant growth and unthrifty plants on D-plot, the ability of these plants to take-up and metabolize sulfur may be reduced.

We intend to also determine the pH, cation exchange capacity, electrical conductivity, and organic matter of the soils. These characteristics have important effects on seed germination.

Western wheatgrass germination and establishment were similar at all fumigation levels, although D-plot values were consistently higher (Figure 13 .4A). Bluebunch wheatgrass responded differently (Figure 13.4B) in that germination and growth occurred much quicker. In this case, the soil with the

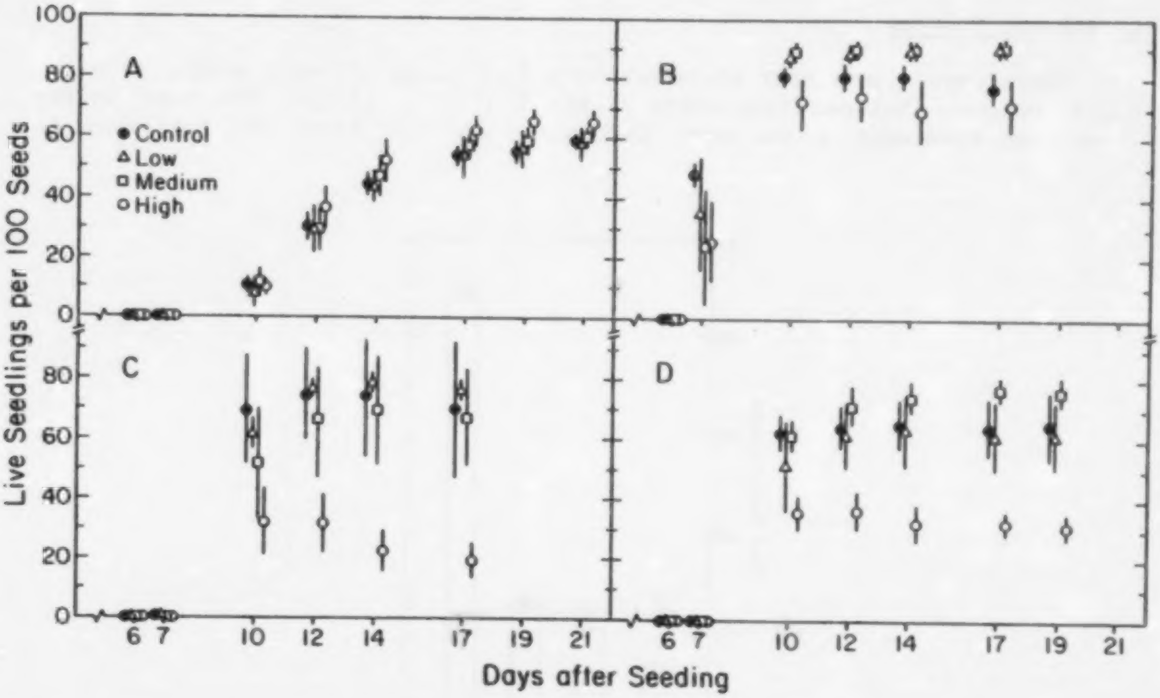

Figure 13.4. Number of live seedlings per 100 seeds; (A) western wheatgrass, (B) bluebunch wheatgrass, (C) sideoats grama, and (D) green needlegrass. Mean ± 1 standard error is shown.

highest level of sulfur enrichment depressed germination. The warm season grass, sideoats grama, showed similar patterns to bluebunch wheatgrass (Figure 13.4C), except that after day 12, seedling mortality increased at each sample date. By day 17, D-plot soil supported an average of 20 plants per 100 seeds, while the A-plot had 70 plants. Green needlegrass germination ceased at day 10 on the D-plot (Figure 13.4D). Seedling loss at the higher sulfur rate was less than observed with sideoats grama. At the end of the study (day 19), the High rate plot had one-half the plants of the Control: 33 vs. 66 per 100 seeds.

The germination rate was significantly lower and seedling mortality higher for wild flax on the D-plot (Figure 13.5A). After day 10 there was a linear decrease in surviving plants, and at day 17 the D-plot supported one-tenth the plant numbers of the other treatments. The other forb, purple prairieclover, reacted similarly to flax (Figure 13.5B), except that the C-plot (Medium sulfur exposure) increased germination and survival compared with all other plots. Germination rate was significantly depressed on the D-plot. Further, this treatment showed a reduction in plant numbers from day 10 through day 17. On the D-plot the highest germination observed was only 12 plants per 100 seeds.

Fourwing saltbush was the only shrub studied (Figure 13.5C), a species characterized with a low germination rate (Eddleman, 1978 and 1979). Even so, the D-plot germination and survival was significantly less than that of the other treatments. By day 17, survival was reduced to three plants per 100 seed, while the Control had 27 plants.

From these species responses, several generalizations can be drawn. The wheatgrass did well in both germination and survival at all sulfur levels. For all other species, germination and survival were reduced on D-plot soils. For no species did any treatment except D significantly lower germination or survival when compared to the A treatment. In some cases B or C plots exceeded A plots in plant survival at the end of the experiment, suggesting a growth enhancement at lower levels of sulfur enrichment. Forbs and the shrub were more adversely affected by SO_2-polluted soils than grasses.

There were no significant differences in number of leaves or heights of seedlings among treatments. It appears that once seedlings establish, growth rate is not affected by high soil sulfur levels and other chemical changes induced by fumigation.

Plant tissue sulfur levels for bluebunch wheatgrass and green needlegrass grown on soils from plots A and D are shown in Figure 13.6. In both species significantly higher sulfur levels were found in the plants from D-plot soils compared to those on the A-plot. This supports the idea that a portion of the high sulfur tissue levels in plants found in areas of SO_2 fumigation comes from the sulfur enriched soils.

Species composition changes, which still are minimal, have appeared on the ZAPS plots only during the past two years. Therefore, the residual seed load in the soil should not differ significantly among treatments. Figure 13.7 illustrates differences in average number of volunteer species (weeds)

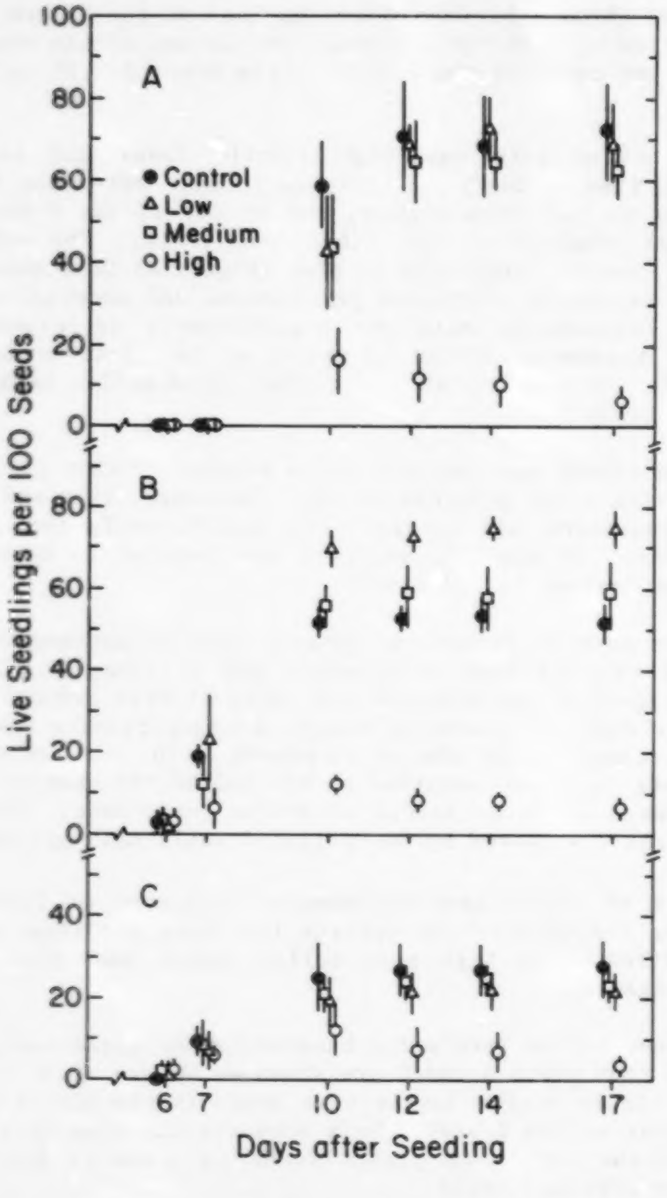

Figure 13.5. Number of live seedlings per 100 seeds; (A) wild flax, (B) purple prairieclover, and (C) fourwing saltbush. Mean ± 1 standard error is shown.

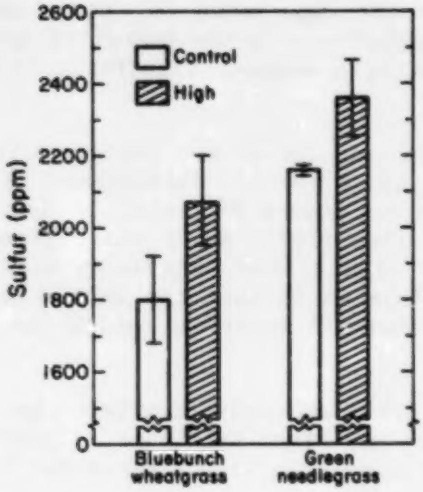

Figure 13.6. Tissue sulfur level for bluebunch wheatgrass and green needlegrass grown on Control and High sulfur soils; mean ± 1 standard error.

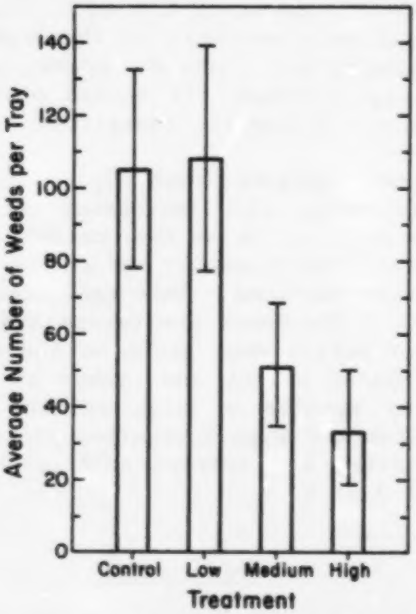

Figure 13.7. Number of volunteer species (weeds) per tray for four levels of fumigated soils; mean ± 1 standard error.

per tray at the end of the experiment for the four levels of fumigation. There is no significant difference in the number of species between the A-plot and the B-plot soils, but both support significantly more species than C and D.

The volunteer plants in the C and D-plot soils germinated much more slowly than those on A- and B-plots. Differences in germination rates were more striking in the earlier phases of the study than at the time of seedling harvest. According to Gordon (1972) seeds which germinate slowly even under favorable conditions are usually the ones which will not survive under more typical circumstances. This is because the favorable period of plant growth in nature usually is short and seedlings which do not become established quickly die.

The diversity index (H') is significantly higher for volunteer plants in the B-plot soils than for any other treatment (Figure 13.8). Values for the D-plot are significantly lower than values for A and B.

Species richness for volunteer plants follows a similar pattern (Figure 13 .9). The B-plot plants are significantly richer floristically than the C and D treatments; D-plot had the fewest volunteer plants.

SUMMARY

Unpolluted soil placed on ZAPS I for 8 weeks showed highly significant linear increases in sulfur levels with the SO_2 fumigation. Soil on the A-plot had significantly less sulfur accumulation than on any of the other treatments. The D-plot soil exceeded all others ($P \leq 0.05$). Green needlegrass seedlings exposed to SO_2 fumigation were necrotic at the high exposure rate. Western wheatgrass, bluebunch wheatgrass, sideoats grama, green needlegrass, purple prairieclover and fourwing saltbush all showed positive linear increases in plant tissue levels with increasing SO_2 fumigation.

Soils which have been fumigated with SO_2 for five growing seasons showed significant increases in sulfur with increasing pollution. The D-plot soils had more than 6 times as much sulfur as the control soils. Germination and seedling establishment were significantly reduced on the D-plot soils for five of the seven native species examined. This reduction was especially dramatic for the forbs and shrub. Bluebunch wheatgrass and green needlegrass showed significant increases in sulfur when grown on the D-plot compared to the A. Growth, represented by plant height and number of leaves, was not significantly affected for any species by high sulfur levels or other chemical changes in the soils from the High fumigation plots. For volunteer plants, species diversity, richness, and average numbers were significantly less on D-plot soils than either A or B.

CONCLUSIONS

The sulfur accumulation reported here probably occurred faster than that which would happen under normal vegetational cover or with episodic rather

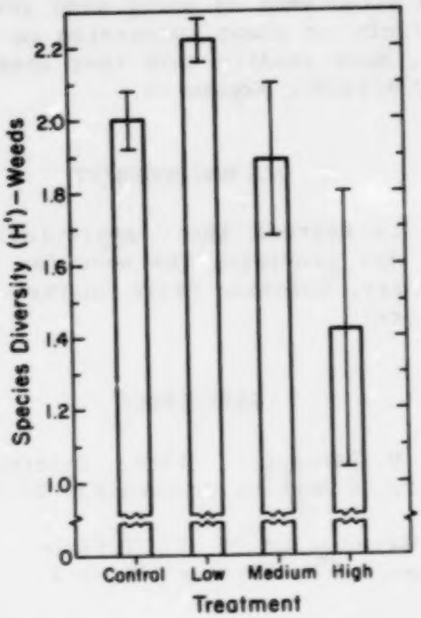

Figure 13.8. Species diversity (H') of volunteer species (weeds) growing in fumigated soils; mean ± 1 standard error.

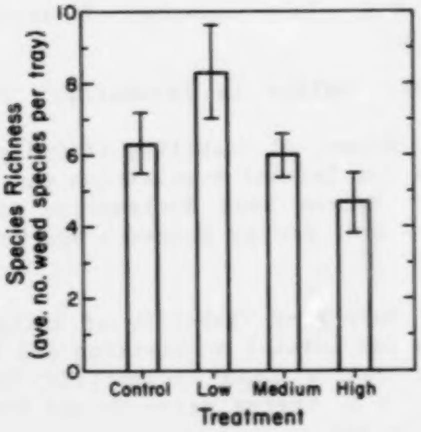

Figure 13.9. Species richness of volunteer species (weeds) growing on fumigated soils; mean ± 1 standard error.

than continuous fumigation. The effects which we observed may have considerable relevance in such situations as strip mine revegetation in the vicinity of coal-fired power plants or plant succession on stressed sites. Also, if soils accumulate sulfur more readily than they lose it, our data may compare with those from chronic episodic exposures.

ACKNOWLEDGEMENT

The authors wish to express their appreciation to Dr. Lee Eddleman, University of Montana, for providing the seed for this study. Also, we are indebted to Mr. Tim McNary, Colorado State University, for watching over the field study in our absence.

REFERENCES

Bardsley, C. E. and J. D. Lancaster. 1960. Determination of Sulfur in Soil. Soil Science Society of America Proceedings 24:265-268.

Bertramun, B. R., M. Fried, and S. L. Tisdale. 1949. Sulfur Status of Indiana Soils. American Fertilizer 111(6):8.

Dodd, J. L., W. K. Lauenroth, G. L. Thor, and M. B. Coughenour. 1979a. Effects of Chronic Low Level SO_2 Exposures on Producers and Litter Dynamics. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 384-393.

Dodd, J. L., W. K. Lauenroth, R. G. Woodmansee, G. L. Thor, and J. D. Chilgren. 1979b. Soil and Meteorological Characteristics at ZAPS. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 331-376.

Eaton and Eaton. 1926. Sulfur in Rainwater. Plant Physiology 1:77-87.

Eddleman, L. E. 1979. Survey of Viability of Indigenous Grasses, Forbs, and Shrubs: Techniques for Initial Acquisition and Treatment for Propagation in Preparation for Future Land Reclamation in the Fort Union Basin. Annual Prog. Rept. U.S. Energy Research and Development Administration. Feb. 1979. 105 pp.

Eddleman, L. E. 1978. Survey of Viability of Indigenous Grasses, Forbs and Shrubs: Techniques for Initial Acquisition and Treatment for Propagation in Preparation for Future Land Reclamation in the Fort Union Basin. Annual Prog. Rept. U.S. Energy Research and Development Administration. Feb. 1978. 183 pp. + App.

Gordon, A. G. 1972. The Rate of Germination. In: Seed Ecology, W. Heydecker, ed. Pennsylvania State Univ. Press. pp. 391-410.

Hanson, D. 1976. Procedure for the Determination of Total Sulfur in Plant Material. Plant Anal. Lab., Oregon State Univ. (mimeo.).

Harper, J. L. 1965. Establishment, Aggression, and Cohabitation in Weedy Species. In: The Genetics of Colonizing Species. H. G. Baker and G. L. Stebbins, eds. Academic Press, New York. pp. 243-265.

Lauenroth, W. K., C. J. Bicak, and J. L. Dodd. 1980. Sulfur Accumulation in Western Wheatgrass Exposed to Controlled SO_2 Concentrations. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report, Colstrip, Montana, E. M. Preston and D. W. O'Guinn, eds. EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon (In Press). pp. 147-152.

McCoal, M. M. and A. Mehlech. 1938. Soil Characteristics in Relation to Distance From Industrial Centers. Cont. from Boyce Thompson Institute 9(4)353-369.

Powers, W. L. ND. The Use of Sulfur in Soils. Texas Gulf Sulfur Comp. Houston, Texas. 89 pp.

Rice, P. M., L. H. Pye, R. Boldi, J. O'Loughlin, P. C. Tourangeau, and C. C. Gordon. 1979. The Effects of "Low Level SO_2" Exposure on Sulfur Accumulation and Various Plant Life Responses of Some Major Grassland Species on the ZAPS Sites. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 494-591.

Rice, P. M., C. C. Gordon, and P. C. Tourangeau. 1980. Weight and Germination Responses of Grass Seeds from Parental Stock Subjected to Sulfur Dioxide Fumigation: In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn, eds. EPA-600/3-80-052. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 153-171.

Shannon, C. and W. Weaver. 1949. Mathematical Theory of Communication. Univ. Illinois Press, Urbana. 117 pp.

Stearns, S. C. 1977. The Evolution of Life History Traits: A Critique of the Theory and a Review of the Data. Ann. Rev. Ecol. Syst. 8:145-171.

Tabatabai, M. A. and J. M. Bremner. 1970. A Simple Turbulemetric Method of Determining Total Sulfur in Plant Materials 62:805-809.

Taylor, J. E., W. C. Leininger, and M. W. Hoard. 1980. Plant Community Succession Studies on ZAPS. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn, eds. EPA-600/3-80-052. U. S. Environmental Protection Agency, Oregon. (In Press). pp. 216-234.

Taylor, J. E. and W. C. Leininger. 1979. Plant Community Changes Due to Low Level SO_2 Exposures. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 610-641.

Treshow, M. 1970. Environment and Plant Response. McGraw-Hill, New York. 422 pp.

Wilson, B. D. 1921. Sulfur Supplied to the Soil in Rainwater. J. Amer. Soc. Agron. 13:226-229.

SECTION 14

RESPONSE OF FIELD POPULATIONS OF SOIL NEMATODES AND ROTIFERS TO THREE LEVELS OF SEASON-LONG SULFUR DIOXIDE EXPOSURE

J. W. Leetham, T. J. McNary, J. L. Dodd, and W. K. Lauenroth

ABSTRACT

Soil nematode and rotifer populations were sampled once in midseason in 1977 and 1978 in two field sites in southeastern Montana, an area included in the northern mixed-grass prairie region. The two field sites were each divided into four 0.52 ha plots and fumigated with low-levels of SO_2 throughout the growing seasons of 1975-1979 (Site I) and 1976-1979 (Site II). No significant treatment effects were found for the rotifers or three trophic groupings of the nematodes (herbivores, saprophages, and predators). With the exception of predatory nematodes, significantly higher populations of all groups were found in the 0-10 cm soil layer than in the 10-20 cm layer.

INTRODUCTION

Soil nematodes and rotifers are ubiquitous in soils of native grassland ecosystems and are considered to have important roles in energy flow and nutrient cycling processes. There is essentially no published information on the effects of air pollutants and specifically SO_2 on the activities of these organisms. There have been a few studies on the effects of soil acidification on enchytraeids and microarthropods (Bååth *et al.*, 1980; Abrahamsen *et al.*, 1978). Artificial acidification caused reduced enchytraeid populations and generally increased Collembola populations. Soil mite (Acarina) populations were unaffected by the changes in soil pH. Sulfur dioxide has been shown to be toxic to soil microorganisms involved in decomposition (Babich and Stotzky, 1974; Bååth *et al.*, 1980; Abrahamsen *et al.*, 1978; Grant *et al.*, 1979; Bryant *et al.*, 1979).

MATERIALS AND METHODS

Each of the treated plots within the two field sites was divided into two equal replicates. Both sites were sampled once in midseason in 1977 (14 July) and again in 1978 (24 July). On each sampling date, five 5.0 cm diameter by 20.0 cm deep soil cores were taken randomly on each replicate. Samples were taken a minimum of 2 m from the delivery pipes. In 1978 a fifth treatment was created by sampling directly beneath the delivery orifices on the High treatment plots. The actual SO_2 concentration exposure at these points was not measured but was considered to be at least double the concentration away from the pipes. The soil cores were divided into 10 cm increments for extraction of nematodes and rotifers. Extraction was by the Baermann funnel technique. All extracted material was preserved in a 1 percent formalin solution. Taxonomic identification of the nematodes and rotifers was not made, only a crude categorization was made based on the presence or absence of stylets. Stylet bearing types were considered largely herbivorous and nonstylet bearers were considered largely saprophagous, although the stylet bearers included some predatory and some fungal feeding nematodes. The only predator separated out was of the genus *Mononchus*. Only total counts of rotifers were made.

RESULTS

Nematode populations varied across sites and years (Figure 14.1). Site I had higher populations on all plots in 1978 while the population size on Site II was about the same in both years. Treatment effects were not apparent and statistical tests were not performed on the total nematode population estimates.

A split plot ANOVA was performed on the 1978 nematode trophic group (herbivores, saprophages, predators) data to test for treatment and depth effects. Since preliminary analysis indicated a linear relationship between means and standard deviations for the saprophages, these data were transformed [LN(X+1)] prior to the ANOVA. For the other trophic groups analyses were performed on untransformed data. A significant treatment effect ($P = .008$) was found only for the log transformed saprophagous nematodes on Site II where there were significantly more saprobes on the High (7.5 pphm) treatment than on the Control (Figure 14.2). All groups showed significant depth differences. For the herbivores and saprophages, there were significantly higher densities in the 0-10 cm level than the 10-20 cm level (Table 14.1). There were higher densities of predators in the 10-20 cm layer. The depth distribution data for 1977 were essentially the same as in 1978 except an even higher percentage (over 90 percent) of the predators was in the 10-20 cm layer.

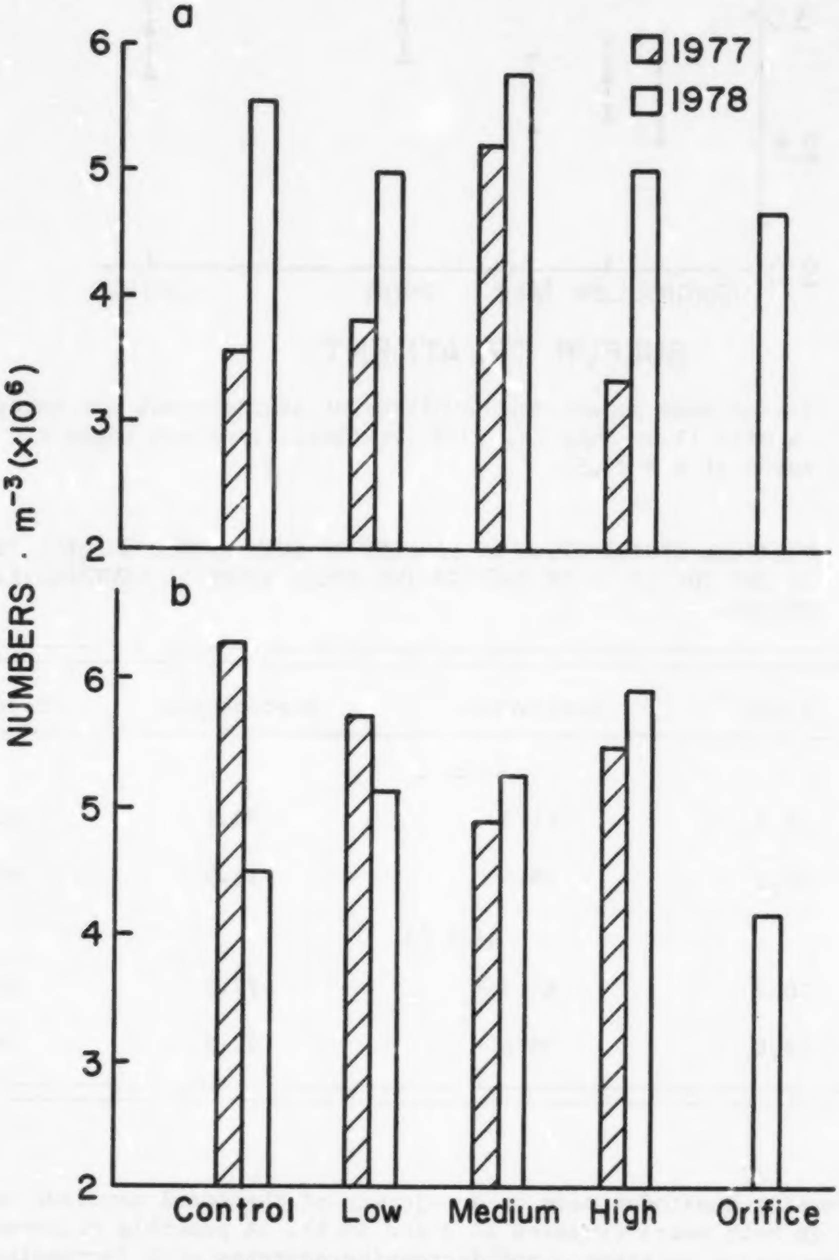

Figure 14.1. Nematode densities in the top 20 cm of soil on two sites in southeastern Montana. A = Site I, B = Site II.

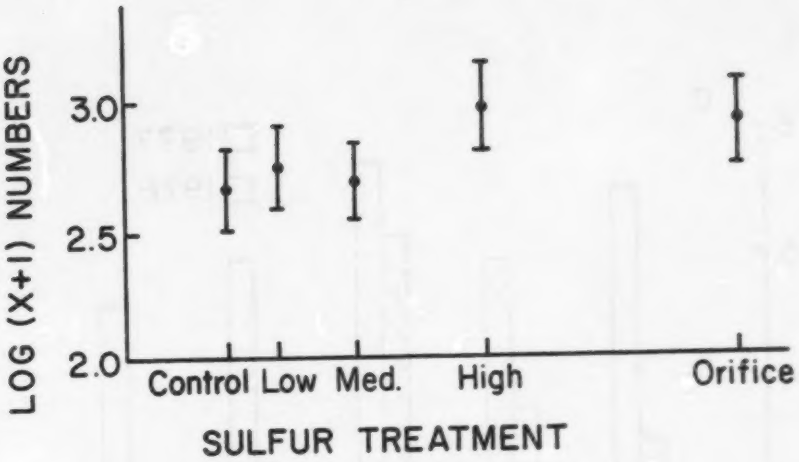

Figure 14.2. Season mean population densities of saprophagous nematodes on Site II on July 24, 1978 (vertical lines are Tukey's Q value at x = 0.05).

TABLE 14.1. VERTICAL DISTRIBUTION BY PERCENT OF SOIL NEMATODE POPULATIONS IN THE TOP 20 CM OF SOIL ON TWO FIELD SITES IN SOUTHEASTERN MONTANA

Depth	Total	Herbivores	Saprophages	Predators
		Site I		
0-10 cm	74.4	71.5	83.1	12.0
10-20 cm	25.6	28.5	16.9	88.0
		Site II		
0-10 cm	70.2	64.4	77.2	43.2
10-20 cm	29.8	35.6	22.8	56.8

Stylet-bearing nematodes made up a majority of the total nematode numbers on both sites in both years (Figures 14.3 and 14.4). A possible treatment response of increasing herbivores and decreasing saprobes with increasing SO_2 exposure occurred on Site I in 1977, but was unsupported by either the 1978 data or the 1977 and 1978 Site II data.

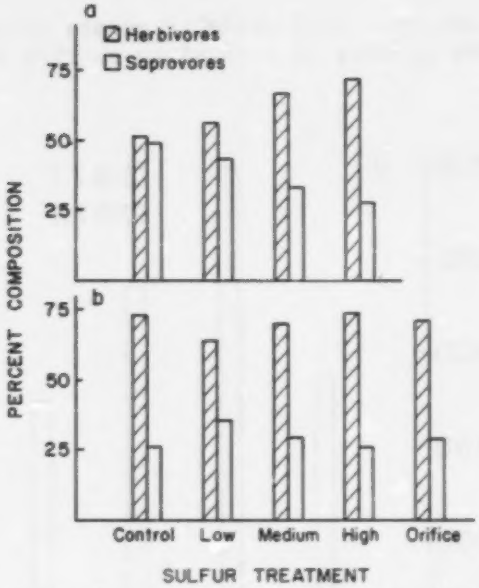

Figure 14.3. General nematode population structure at Site I by percent composition. Predators were not included because they were negligible by comparison. A = 1977, B = 1978.

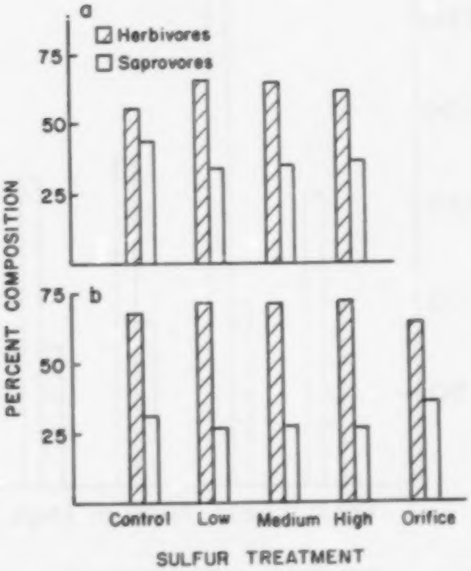

Figure 14.4. General nematode population structure at Site II by percent composition. Predators not included because they were negligible by comparison. A = 1977, B = 1978.

Rotifer populations were also variable across sites and years (Figure 14.5). Densities were greater on both sites in 1978 than in 1977 with Site I

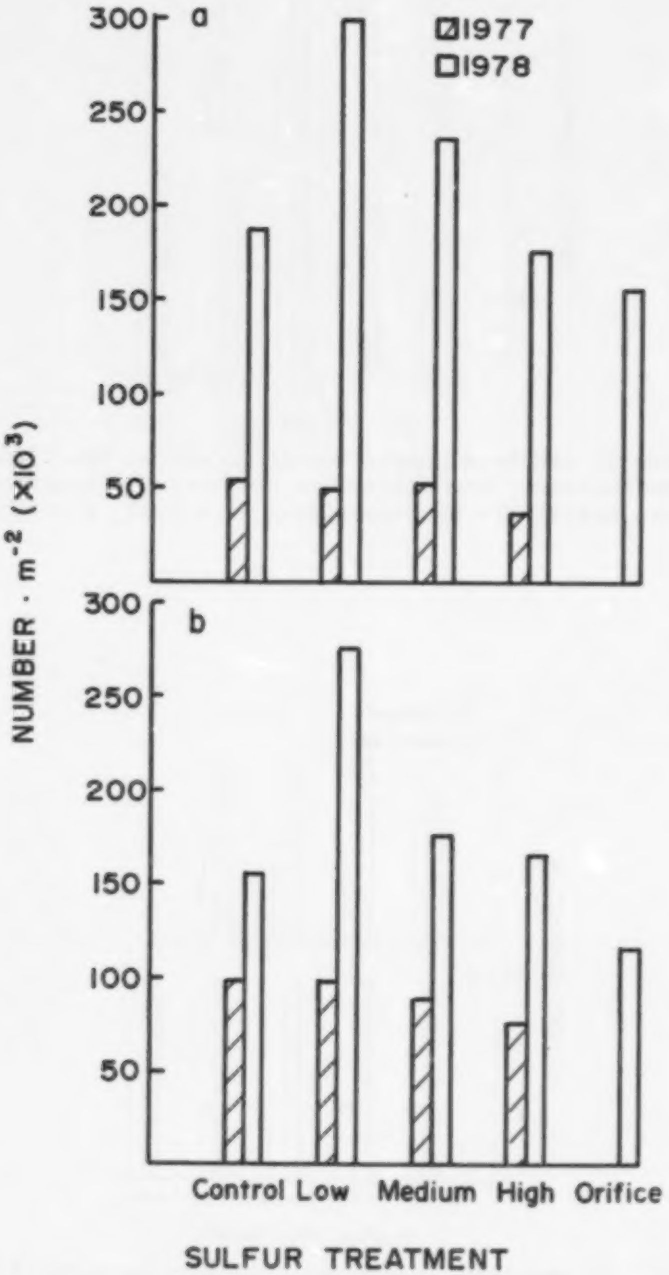

Figure 14.5. Rotifer densities in the top 20 cm of soil on two sites in southeastern Montana. A = Site I, B = Site II.

showing the greatest increase. The site and year differences were not tested statistically, however the 1978 data were tested with a split plot ANOVA for treatment and depth differences. No significant treatment differences were found on either site, however, significant depth differences were found on both sites ($P<.01$). The average proportions of rotifer populations in the 0-10 cm layer was 91 percent and 92 percent for Site I and II respectively. The figures for 1977 were essentially the same, *i.e.*, 91 percent and 87 percent for Site I and II respectively.

DISCUSSION

With the exception of the increased saprophagous nematode populations on the high treatment of Site II in 1978, significant treatment responses were not found in either nematodes nor rotifers. This suggests that the SO_2 exposure of the field plots did not have any overall measurable effects on either group. However, since species identifications were not made, it is not known if any species may have responded to the exposure but their population changes were masked in the total counts. The one case of significantly higher saprophagous nematodes in the High treatment plot of Site II appears not to be representative of any trend since none of the other data sets support the finding. It appears to be a chance occurrence, attributable to the variability of the data. If, indeed, the higher saprophagous densities were due to SO_2 exposure, the reasoning behind such a shift might be that the nematodes increased in response to decreased microbial activity which is suspected from other studies (Leetham *et al.*, 1980; Dodd *et al.*, 1980). The nematodes could conceivably be utilizing a resource left available by the decreased microbes.

The higher nematode and rotifer densities near the soil surface are expected since the plant root biomass is similarly distributed (Lauenroth *et al.*, 1975). The lack of significant treatment effects on the nematodes and rotifers can be explained by either a lack of sensitivity or a lack of contact with SO_2 or its derivatives. The question of sensitivity to SO_2 cannot be commented on since there is no published information on the effects of SO_2 on either soil nematodes nor rotifers. We suspect that the relatively low level of SO_2 exposure to the soil surface did not result in any significant chemical changes below the surface because of the buffering capacity of the soil. Leetham *et al.* (1980) have shown that tardigrade populations in the upper 2 cm of the soil profile of these same plots were reduced by low-level SO_2 exposure. For subsurface soil organisms, time may be the critical factor in whether or not chronic low-level SO_2 exposure will have an effect on their population size and/or function.

CONCLUSIONS

No significant large scale changes occurred in field populations of soil nematodes and rotifers in the experimentally fumigated plots with the possible exception of an increase in nonstylet bearing (largely saprophagous) nematodes on the high treatment plot of Site II in 1978. However, the level of identification used in this study precluded the detection of responses in particular species or genera. Significantly higher populations of both nematodes and rotifers occurred in the top 10 cm of the soil profile as compared to the 10-20 cm level. The buffering capacity of the soil is thought to reduce or eliminate the effects of SO_2 and/or its derivatives on the soil dwelling invertebrates that occur below the surface 1 or 2 cm.

REFERENCES

Abrahamsen, G., T. Hovland, and S. Hagvar. 1978. Effects of Artificial Acid Rain and Liming on Soil Organisms and the Decomposition of Organic Matter. SNSF-contribution FA 28/78. 23 pp.

Bååth, E., B. Berg, U. Lohm, B. Lundgren, H. Lundkvist, T. Rosswall, B. Söderström, and A. Wiren. 1980. Effects of Experimental Acidification and Liming on Soil Organisms and Decomposition in a Scots Pine Forest. Pedobiologica.

Babich, H., and G. Stotzky. 1974. Air Pollution and Microbial Ecology. Crit. Rev. Environ. Control, 4:353-421.

Bryant, R. D., E. A. Gordy and E. J. Laishley. 1979. Effect of Soil Acidification on the Soil Microflora. Water, Air, and Soil Pollut., 11:437-445.

Dodd, J. L. and W. K. Lauenroth. 1980. Effects of Low-level SO_2 Fumigation on Decomposition of Western Wheatgrass Litter in a Mixed-grass Prairie. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn (eds.) EPA-600/3-80-052, U. S. Environmental Protection Agency, Corvallis, Oregon. (In Press) pp. 212-215.

Grant, I. F., K. Bancroft, and M. Alexander. 1979. SO_2 and NO_2 Effects on Microbial Activity in an Acid Forest Soil. Microbial Ecology, 5:85-89.

Lauenroth, W. K., J. L. Dodd, R. K. Heitschmidt, and R. G. Woodmansee. 1975. Biomass Dynamics and Primary Production in Mixed Prairie Grasslands in Southeastern Montana: Baseline Data for Air Pollution Studies. Fort Union Coal Field Symposium, Eastern Montana College, Billings. pp. 559-578.

Leetham, J. W., T. J. McNary, J. L. Dodd, and W. K. Lauenroth. 1980. Response of Field Populations of Tardigrada to Various Levels of Chronic Low-level Sulfur Dioxide Exposure. Proceedings of VII International Colloquim of Soil Zoology.

Lewis, R. A., A. S. Lefohn, and N. R. Glass. 1976. Introduction to the Colstrip, Montana, Coal-fired Power Plant Project. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Second Interim Report, Colstrip, Montana. R. A. Lewis, A. S. Lefohn, and N. R. Glass (eds.) EPA-600/3-76-013, Environmental Protection Agency, Corvallis, Oregon. pp. 1-12.

SECTION 15

ARTHROPOD POPULATION RESPONSES TO THREE LEVELS OF CHRONIC SULFUR DIOXIDE EXPOSURE IN A NORTHERN MIXED-GRASS ECOSYSTEM

I. SOIL MICROARTHROPODS

J. W. Leetham, J. L. Dodd, R. D. Deblinger, and W. K. Lauenroth

ABSTRACT

The effects of season-long exposure of soil microarthropods to controlled levels of SO_2 were investigated under field conditions from 1975 to 1978 in southeastern Montana. Two field sites wherein three levels of SO_2 were dispersed on 0.52 ha plots of grassland communities were used. Periodic sampling was done throughout the growing seasons of 1975 and 1976 while only single, mid-season samplings were made in 1977 and 1978. Soil microarthropods were sampled by taking soil cores and extracting them in high-temperature gradient Tullgen extractors. Large scale population changes among the microarthropods, as a whole were not observed, however significant population reductions were observed for some groups. The most notable reductions occurred in the Collembola where three families (Poduridae, Entomobryidae-Isotomidae, and Sminthuridae) had significant population reductions in the second year of SO_2 exposure on Site I. Only a few acarine families showed significant population reductions. However, the affected groups included representatives of three main trophic or functional groups among the microarthropods - herbivores, fungivores, and predators. The data suggest that the greatest effects of SO_2 occurred during the first half of the growing season when soil water content and population levels were highest. The implications of the population reductions are discussed.

INTRODUCTION

Studies of the effects of air pollutants on soil microarthropods are very few. Most studies of pollutants and soil biota have dealt with microflora and microfauna (bacteria, fungi, etc.). Lebrun *et al.* (1977) found a bark-living oribatid mite, *Humerobates rostrolamellatus* (Grandjean) very sensitive to SO_2 exposure. A concentration of 45 pphm (585 $\mu g \cdot m^{-3}$) for 48 hours produced 50 percent mortality. A followup study (Lebrun *et al.*, 1978) found that relative humidity had a substantial effect on the sensitivity of the oribatid species to SO_2. Mortality rates were significantly increased when the relative humidity was increased from 20 percent to 80 percent while SO_2 concentration was held constant. As will be noted later in this paper, the oribatid mites compose a large portion of the soil microarthropod fauna on our field sites.

Studies of artificial acidification of pine forest soils in Norway (Abrahamsen *et al.*, 1978) and Sweden (Bååth *et al.*, 1980) found increased collembolan populations but only minor changes in the soil acarines. Abrahamsen *et al.*, found two oribatid species were significantly reduced while Bååth *et al.* found some oribatid population reductions, but none were significant. Both studies found populations of mesostigmatid mites were reduced but not significant. Also in both studies, the enchytraeid *Cognettia sphagnetorum* (Vejd.) was severely reduced in acidified plots. The fact that both studies were conducted in pine forest soils which are normally acidic may account for the increased collembolan populations due to adaptation to the acidic conditions. These studies are relevant here since SO_2 can cause acidification of soils, especially in the surface 1 to 2 cm layer where most microarthropods reside.

MATERIALS AND METHODS

Each of the treated plots within the two field sites was divided into two equal replicates from which random samples were taken. All samples were taken a minimum of 2 m from the delivery pipes (except for additional samples taken beneath the pipes in 1977 and 1978). Soil microarthropods were sampled by taking 4.8 cm diameter by 5.0 cm deep soil cores with a coring tool designed to minimize compaction and at the same time retain the sample in a 5 cm long aluminum sleeve. Vegetation cover was clipped to crown level prior to sampling but surface litter was retained on the surface of the core. A Macfadyen-type high temperature gradient Tullgren extraction system was used to retrieve the arthropods from the soil samples (Merchant and Crossley, 1970). The extraction period was 7 days. The arthropods were killed and preserved in 70 percent ethyl alcohol and later counted and identified with a binocular dissecting microscope. Identification was to family and where possible to genus, but occasionally only to order. Identifications below family were hampered by the abundance of undescribed species, especially among the Acarina. For this study, "soil microarthropods" included the soil acarines, apterygote insects, pauropods, symphylids and tardigrades. Tardigrades are treated in detail in another paper (Leetham *et al.*, 1979). Representatives of various groups were dried at 60°C for 48 hours and weighed for dry weight biomass.

Site I was sampled 6 times during the 1975 and 1976 seasons while Site II was sampled 6 times in the 1976 season. Both sites were sampled once in midseason in 1977 and Site I was sampled once in midseason in 1978. Sampling intensity for 1975 and 1976 was five samples per replicate (10 per treatment) per date. Sample numbers were doubled in the 1977 and 1978 seasons (10/replicate). Also in 1977 and 1978, an additional treatment level was created by sampling directly beneath the delivery orifices on the High treatment plot. The SO_2 concentration at those points was not measured but was considered to be at least double the concentration elsewhere on the plot.

A split-plot ANOVA was performed on the data to test for significant treatment and date-within-year effects. Because of the design of the field experiment, we concluded that it would be illogical to include "sites" and "years" as variables in the ANOVA. Therefore, individual ANOVA's were performed by group by year (or single dates in 1977 and 1978). Since the number of groups (orders and families) was quite large, ANOVA's were performed only on those which displayed obvious trends in population changes with increasing SO_2 fumigation (as judged by season mean density and/or biomass in 1975 or 1976 or single samplings in 1977 and 1978). Both density and biomass were used in the ANOVA's. It should be stated at this point that although the traditional significance level of $P = 0.05$ was used as a guideline in judging population changes as significant, probabilities of $0.05 < P < 0.10$ are also considered, primarily because of the inherently high variability of the field data. They were also used when similar population changes occurred in more than one site-year for the same group. In cases where both treatment effects and date-by-treatment interactions were significant, the interaction was given presidence over the main effect. Tukey's Q procedure was used to calculate least significant ranges to pinpoint where the significant differences occurred.

RESULTS

The count to date of identified microarthropods includes 67 families of Acarina, six families of Collembola, two families of Diplura plus tardigrades, pauropods and symphylans. A large majority of the soil microarthropod community was composed of Acarina (Tables 15.1, 15.2, 15.3) with Collembola being a distant second. All other groups appear to be very minor. Among the Acarina the Prostigmata was by far the largest suborder with Cryptostigmata, Mesostigmata, and Astigmata following in that order. Large population changes in response to SO_2 exposure are not readily apparent among most of the major groups of microarthropods shown in Tables 15.1, 15.2, and 15.3.

Results of the statistical analyses are presented in Tables 15.4 and 15.5. All groups on which individual ANOVA's were performed are included for comparison whether or not they had significant population changes. Also, the results presented are for densities only since the results for biomass were essentially the same. Where differences occurred between density and biomass, they will be discussed in text.

First year fumigation effects among the microarthropods on ZAPS I are evident (Table 15.4) but statistical support is lacking due primarily to high variability of the data. Of the groups that displayed trends of reduced populations in the treated plots, only two were significant. The family Paratydeidae (Prostigmata) presented a peculiar response in that the Low and Medium treatments were significantly reduced from the Control while the High treatment was not (Figure 15.1A). The same trend was found again in 1976, 1977, and 1978 (Figures 15.1B,C,D). Densities and biomass of Oribatulidae (Cryptostigmata) were reduced on all plots (Figure 15.2A). Similar reductions occurred again in 1976, 1977, and 1978 but only those in

TABLE 15.1. SOIL MICROARTHROPOD POPULATION STRUCTURE OF SITE I IN SOUTHEASTERN MONTANA*

	SO_2 Treatment							
	Density (Nos. · m^{-2})†				Biomass (mg · m^{-2})			
Group	Control	Low	Medium	High	Control	Low	Medium	High
1975								
Total Microarthropods	50,900	49,200	47,300	48,800	26.2	24.5	24.5	31.9
Acarina	45,000	42,500	40,900	40,000	19.6	15.6	20.7	18.7
Suborders:								
Mesostigmata	1,800	1,500	1,500	2,300	5.6	2.3	7.9	4.4
Prostigmata	34,100	34,600	32,700	29,600	7.9	7.7	7.7	7.7
Cryptostigmata	8,900	5,900	6,300	7,700	5.9	5.2	4.8	6.3
Astigmata	200	500	400	400	0.2	0.4	0.3	0.3
Diplura	< 100	100	200	300	0.3	1.0	1.6	2.8
Collembola	4,300	4,300	5,300	6,300	4.4	4.5	5.7	7.1
1976								
Total Microarthropods	88,200	86,700	119,200	89,200	49.4	38.3	50.8	40.3
Acarina	70,600	76,600	106,600	79,700	31.1	25.9	34.4	28.4
Suborders:								
Mesostigmata	3,300	3,400	3,100	3,800	9.2	6.2	5.7	9.6
Prostigmata	55,200	64,100	88,700	67,800	12.6	14.0	18.7	14.0
Cryptostigmata	11,700	8,400	13,700	7,500	9.0	5.0	9.1	4.4
Astigmata	400	1,000	1,100	600	0.3	0.7	0.9	0.4
Diplura	100	300	400	200	0.9	2.7	3.6	2.2
Collembola	15,400	8,400	8,300	6,700	14.3	8.0	7.2	6.1

* Figures are season means for the top 5 cm of the soil profile.

† Numbers are rounded to nearest hundred.

1978 were significant (Figure 15.2C). A significant date by treatment interaction occurred in 1976 (Figure 15.3B) indicating the reductions were not significant on the fourth sample date. Three date by treatment interactions were significant, one for the suborder Prostigmata and two for families Pyemotidae and Scutacaridae. For the total Prostigmata, there were significantly reduced densities in the High treatment plot on one sample date (data not shown). However, the same trend was not evident on any other date. The two families, Pyemotidae and Scutacaridae, were quite similar in that significant treatment differences occurred only in the spring and early summer (Figure 15.4) when they were most abundant on the field plots. During the remainder of the season their densities were negligible. In both cases, densities were significantly reduced in the High treatment plots.

Collembolan populations were significantly affected by SO_2 on Site I in 1976 (Figures 15.5 and 15.6). Total collembolan density was reduced on all

TABLE 15.2. SOIL MICROARTHROPOD POPULATION STRUCTURE AS MEASURED IN MID-GROWING SEASON IN 1977 AND 1978 ON SITE I[*]

	SO_2 Treatments									
	Density (Nos. · m^{-2})[†]					Biomass (mg · m^{-2})				
Group	Control	Low	Medium	High	Orifice	Control	Low	Medium	High	Orifice
10 July 77										
Total Microarthropods	84,800	111,000	134,600	65,000	84,800	33.4	36.6	51.8	29.0	37.1
Acarina	82,600	108,900	132,600	64,200	83,400	29.8	33.5	49.5	27.8	35.8
Suborders:										
Mesostigmata	2,500	3,500	4,600	3,500	6,300	7.2	7.7	9.7	8.3	12.9
Prostigmata	71,100	90,700	110,000	52,300	65,200	14.9	17.3	24.4	11.8	14.7
Cryptostigmata	8,000	12,300	16,500	82,000	10,900	7.0	6.7	14.3	7.6	7.7
Astigmata	1,000	2,400	1,400	200	1,000	0.7	1.8	1.1	0.1	0.5
Diplura	<100	0	0	0	0	0.3	0.	0.	0.	0.
Collembola	500	300	600	200	500	0.2	0.1	0.1	0.3	0.3
8 July 78										
Total Microarthropods	97,100	69,400	70,700	72,700	59,500	39.6	27.5	25.3	30.8	21.9
Acarina	95,400	68,300	68,900	71,300	58,000	36.8	26.0	23.2	28.6	19.7
Suborders:										
Mesostigmata	1,500	2,200	1,300	2,300	2,100	3.9	5.7	1.9	4.7	4.1
Prostigmata	74,100	53,400	51,400	57,900	45,700	16.8	9.9	10.9	12.1	9.1
Cryptostigmata	19,400	12,500	16,100	10,600	10,300	15.8	10.2	10.3	11.5	6.5
Astigmata	400	200	200	400	<100	.3	.2	.1	.3	<.1
Diplura	100	<100	<100	100	100	.8	.5	.3	.8	1.3
Collembola	800	400	900	500	900	.8	.2	.7	.3	.4

[*] Figures are summaries for the top 5 cm of the soil profile.

[†] Numbers are rounded to nearest hundred.

treated plots, however, for biomass, a significant treatment-by-date interaction resulted due to populations on all plots being similar late in the growing season. Treatment differences occurred during the first half of the season. Within the Collembola, both density and biomass of Poduridae were significantly reduced on the treated plots (only biomass data are presented in Figure 15.6). Significant treatment-by-date interactions for density and biomass occurred for both the Entomobryidae and Sminthuridae. Members of the family Isotomidae were included in the Entomobryidae. Since the entomobryids accounted for a majority of the total Collembola, the similarity of Figures 15.5B and 15.6B is expected. There were reduced populations of Sminthuridae on all the treated plots on four of six sample dates (Figure 15.6C).

Although density and/or biomass reductions occurred on one or more treated plots on Site I in many acarine groups after 1975, only a few of

TABLE 15.3. SOIL MICROARTHROPOD POPULATION STRUCTURE OF SITE II IN SOUTHEASTERN MONTANA*

	SO_2 Treatments									
	Numbers · m^{-2}					Biomass (mg · m^{-2})				
Group	Control	Low	Medium	High	Orifice†	Control	Low	Medium	High	Orifice†
A-1976 Season Summary										
Total Microarthropods	85,300	76,200	130,100	93,700	-	42.8	41.1	52.1	47.4	-
Acarina (Total)	71,200	58,100	94,600	76,700	-	28.9	22.4	34.5	27.8	-
Suborders:										
Mesostigmata	2,600	2,400	3,000	2,700	-	5.8	6.4	5.1	4.1	-
Prostigmata	58,500	48,000	77,200	62,600	-	12.6	10.1	15.7	13.3	-
Cryptostigmata	9,000	7,400	13,400	10,600	-	9.7	5.6	12.9	9.8	-
Astigmata	1,100	300	1,000	800	-	0.8	0.3	0.8	0.6	-
Diplura	100	100	200	200	-	1.2	0.9	1.9	1.5	-
Collembola	11,700	15,600	12,000	12,400	-	10.0	14.7	11.2	11.5	-
7 August 77 (One Date)										
Total Microarthropods	63,700	94,300	143,300	103,500	49,200	24.8	37.1	81.9	47.2	17.7
Acarina	62,100	92,200	121,000	99,700	46,900	22.7	34.2	47.7	41.8	14.0
Suborders:										
Mesostigmata	3,400	3,900	6,100	6,600	2,100	4.0	5.1	13.0	10.1	2.4
Prostigmata	50,800	77,300	100,300	79,400	39,700	11.6	17.1	20.2	18.3	8.0
Cryptostigmata	7,700	10,800	14,400	13,000	5,000	6.9	11.9	14.3	12.8	3.5
Astigmata	200	200	200	700	100	.2	.1	.2	.6	.1
Diplura	0	<100	<100	<100	200	0	.3	.1	.5	1.8
Collembola	300	600	800	1,700	1,500	.3	.3	.7	1.5	1.1

* Figures are for the top 5 cm of the soil profile.

† Sampled only in 1977 (see text).

those reductions were significant. Tectocepheidae (Cryptostigmata) density and biomass were reduced on the treated plots in 1976 (density data are presented in Figure 15.3). The little-known family Pediculochelidae (Prostigmata) had significantly reduced densities and biomass in all treated plots on 10 July 77 and a closely related but undescribed family (unknown endeostigmatid, probably represented by just one species on the Sites) also had reduced densities and biomass on all treated plots on 10 July 1977. Two predatory families, Eupodidae and Rhagidiidae (both Prostigmata) had significantly reduced densities and biomass on all treated plots on 8 July 1978. The plant-feeding spider mites, Tetranychidae (Prostigmata), had reduced densities and biomass on all treated plots on 8 July 1978. Density data for the last five families mentioned are presented in Figure 15.7.

TABLE 15.4. ANOVA RESULTS FOR SOIL MICROARTHROPOD DENSITIES ON ZAPS I

	1975 Season		1976 Season		10 July 77	8 July 78
Group	Trt*	D x T†	Trt	D x T	Trt	Trt
Collembola			.062	-		
Entomobryidae			-	.067		
Poduridae			.016	-		
Onychiuridae	-‡	-				
Smintburidae			-	.006		
Protura						
Eosentomidae			-	-		
Symphyla	-	-				
Acarina						
Astigmata						
Acaridae					-	
Prostigmata	-	.003				-
Alicorhagiidae	-	-			-	-
Anystidae			-	-	.016	-
Bdellidae						-
Cryptognathidae						-
Cunaxidae	-	-	-	.093		
Unknown Endostigmatid					.027	-
Eriophyidae						-
Eupodidae	-	-	-	-		.001
Nanorchestidae	-	-				-
Paratydeidae	.058	-	.015	-	.009	.001
Pediculochelidae			-	-	.066	
Pyemotidae	-	<.001			-	
Raphignathidae						-
Rhagidiidae					-	.007
Scutacaridae	-	.004				-
Tarsonemidae					-	-
Tenuipalpidae					-	
Tetranychidae						.001
Cryptostigmata	-	-				
Brachychthoniidae	-	-			-	-
Ceratozetidae			-	-		
Gehypochthoniidae			-	-		
Haplozetidae			-	-		
Immatures (Total)			-	-		
Oppiidae	-	-	-	.050		
Oribatulidae	.073	-	-	.006	-	.001
Sphaerochthoniidae			-	-		
Tectocephidae			.018	-		-

* Treatment main effect.

† Treatment by date interaction.

‡ Indicates the group showed a trend of season mean population reduction with SO_2 exposure, but reductions were not significant at $P \geq .10$. Blank spaces indicate no trends and hence not tested statistically.

Three significant treatment-by-date interactions occurred for the families Oppiidae (Cryptostigmata), Oribatulidae (previously mentioned), and Cunaxidae (Prostigmata). All three families showed consistent trends of reduced populations in the treated plots with the greatest reductions occurring in the high treatment (Figure 15.3). A significant treatment effect occurred for the prostigmatid family Anystidae, however the trend bore no

TABLE 15.5. ANOVA RESULTS FOR SOIL MICROARTHROPOD DENSITIES ON ZAPS II

	1976 Season		7 Aug 77
Group	Trt*	D x T†	Trt
Collembola			
Entomobryidae	-‡	-	
Sminthuridae	-	.013	
Pauropoda	-	-	
Acarina			
Astigmata	-	-	
Acaridae	-	-	
Hypopi	-	-	
Prostigmata			
Alicorhagiidae			-
Unknown Endostigmatid			-
Erythraeidae			-
Eupodidae	-	.003	
Nanorchestidae	.045	-	.001
Nematalycidae	-	-	
Pyemotidae			-
Raphignathidae	-	-	
Scutacaridae	-	.063	
Tenuipalpidae			-
Trombidiidae	.040	-	
Cryptostigmata			
Ceratozetidae	-	-	-
Immatures (Total)			-
Galumnidae	-	-	
Scutoverticidae	.047	-	-
Tectocepheidae			-
Mesostigmata			
Laelapidae	-	.017	

* Treatment main effect.

† Treatment by date interaction.

‡ Indicates the group showed a trend of season mean population reduction with SO_2 exposure, but reductions were not significant at $P \gtrsim .10$. Blank spaces indicate no trends and hence not tested statistically.

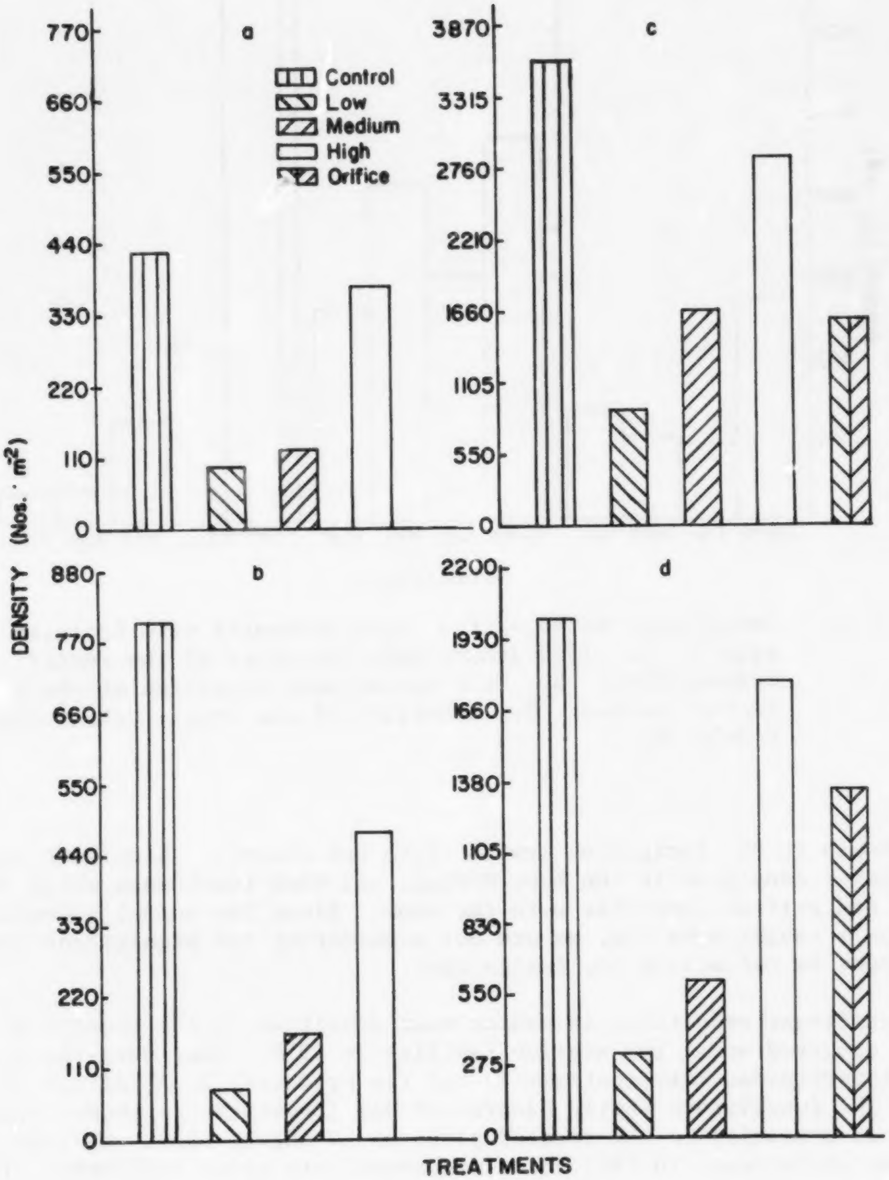

Figure 15.1. Density estimates for the family Paratydeidae on ZAPS I. A. 1975 season means. B. 1976 season means. C. 10 July 77. D. 8 July 78.

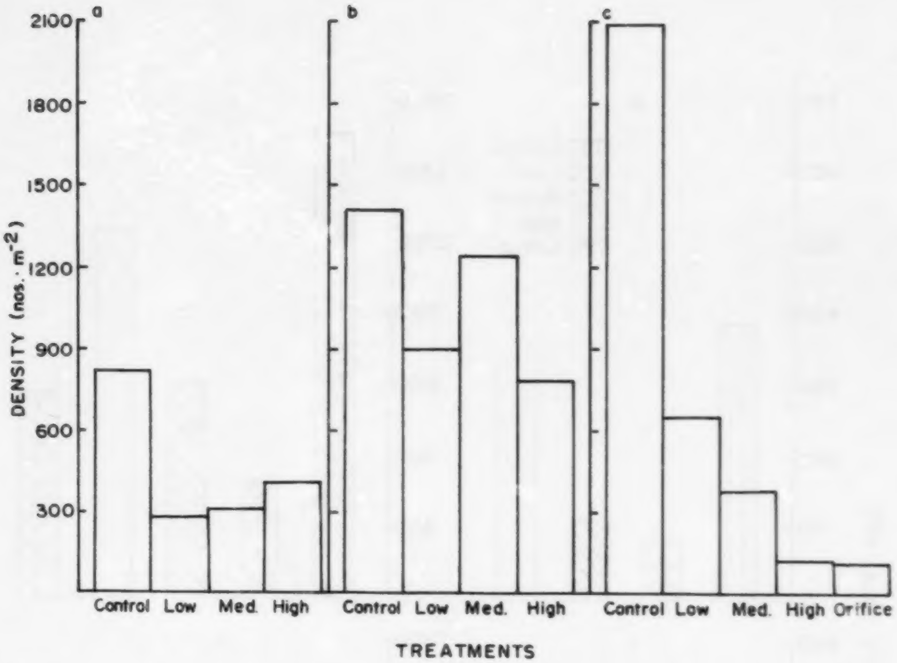

Figure 15.2. Density estimates of two cryptostigmatid mite families on Site I. A. 1975 season mean densities of the family Oribatulidae. B. 1976 season mean densities of the family Tectocepheidae. C. Densities of the family Oribatulide on 8 July 78.

relationship to SO_2 fumigation levels (data not shown). There were significantly lower densities in the Low, Medium, and High treatments while the Control and orifice densities were the same. Since the actual numbers of individuals caught were low, we are not considering the significant treatment differences as reflecting SO_2 fumigation.

Significant reductions in season mean densities on the treated plots on Site II occurred among two acarine families in 1976. They were the fungivorous Scutoverticidae (Cryptostigmata) and the predatory Trombidiidae (Prostigmata). The fungivorous family Nanorchestidae (Prostigmata) showed significantly reduced densities on the treated plots on 10 August 1977. However the treatment differences in 1976 (data not shown) are quite different. In that year there was significantly greater densities and biomass in the Medium treatment than the other treatments and Control which were all essentially the same. Data for these three families are shown in Figure 15.8. Significant treatment-by-date interactions occurred among two acarine families on Site II in 1976. They included the predatory Eupodidae and the functionally unknown Scutacaridae (both Prostigmata). In both cases, treatment differences occurred early in the growing season at which time densities were reduced on all treated plots, especially the High treatment plot (Figure 15.9). A

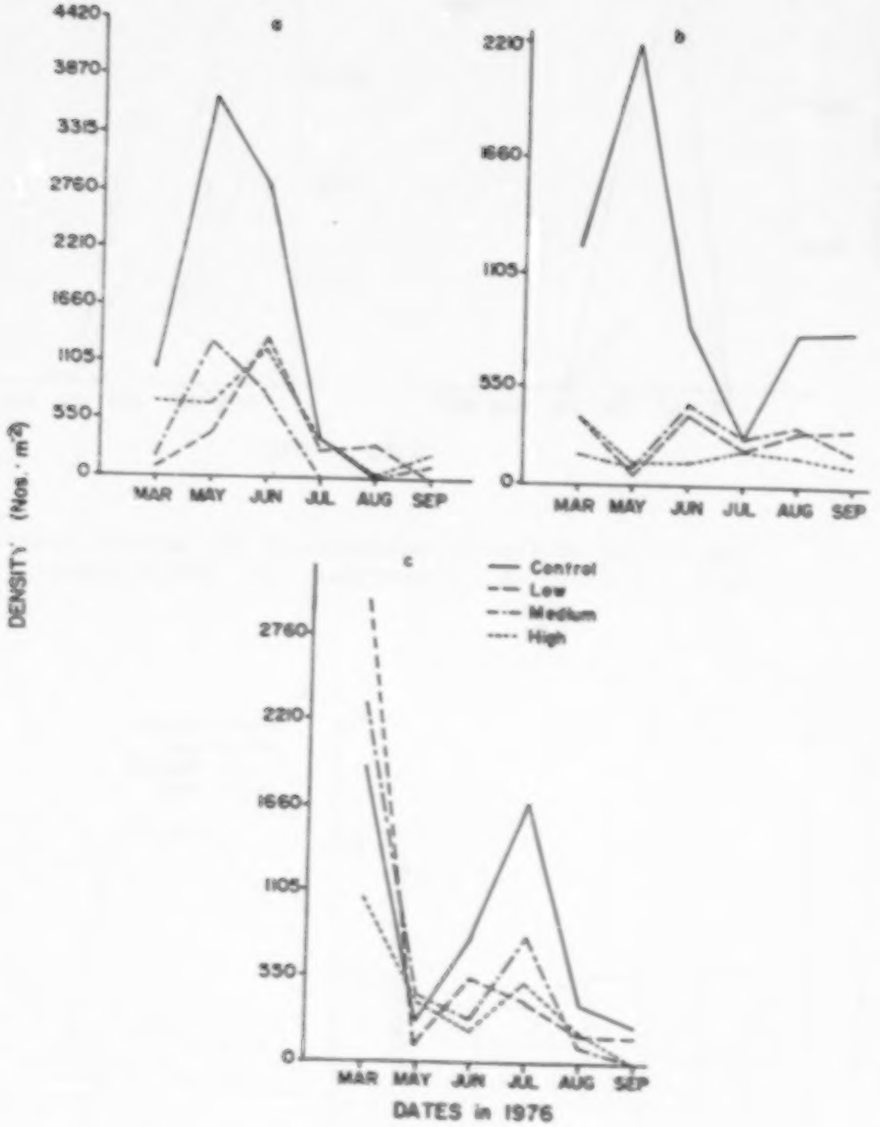

Figure 15.3. Time traces of density estimates of three acarine families on ZAPS I in 1976. A. Oppiidae. B. Oribatulidae. C. Cunaxidae.

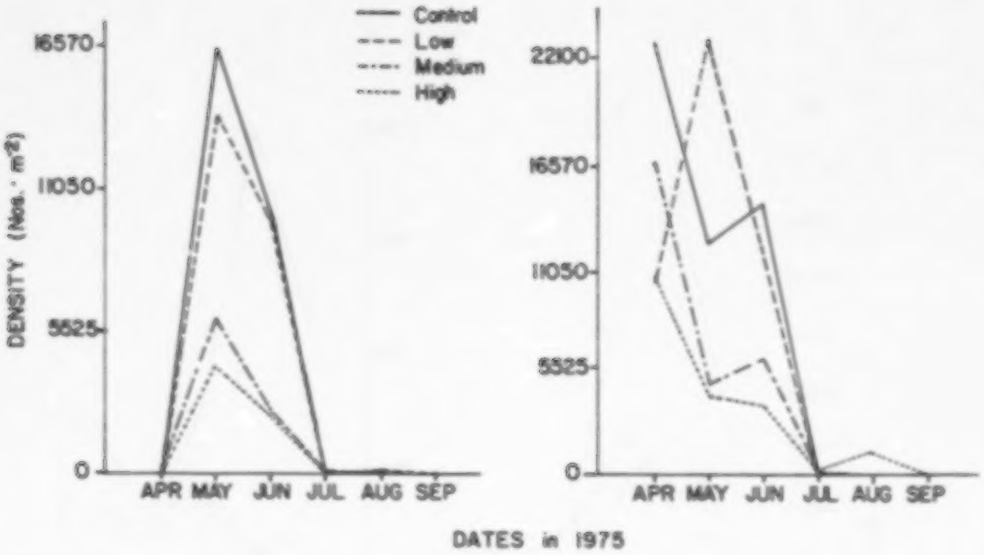

Figure 15.4. Time traces of density estimates of two acarine familes on ZAPS I in 1975. A. Pyemotidae. B. Scutacaridae.

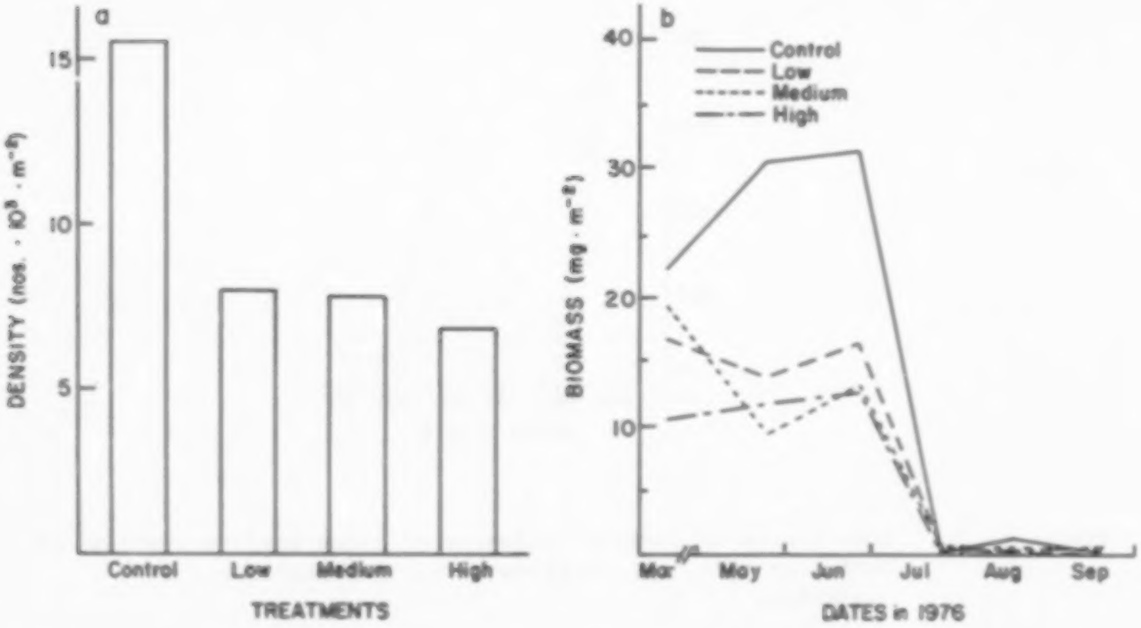

Figure 15.5. Density and biomass estimates of Collembola (total) on Site I in 1976. A. Season mean densities. B. Time traces of biomass.

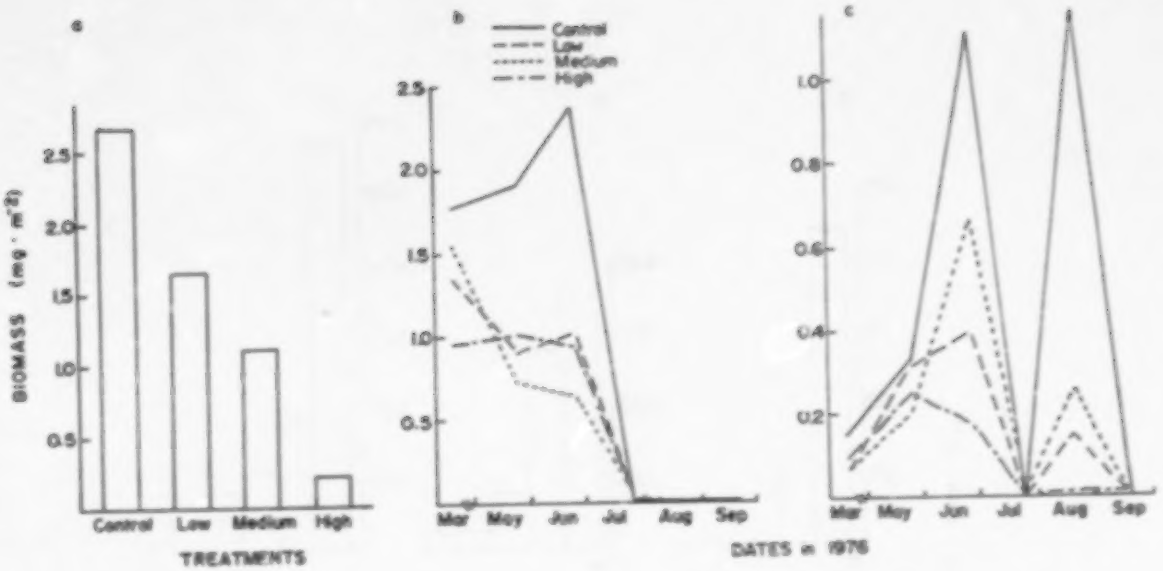

Figure 15.6. Biomass dynamics of three collembolan families on Site I in 1976. A. Season mean biomass estimates of Poduridae. B. Time traces of biomass estimates of Entomobryidae - Isotomidae. C. Time traces of biomass estimates of Sminthuridae.

significant treatment-by-date interaction occurred for Sminthuridae (Figure 15.10) where the High treatment was significantly reduced from the Control on the first sample date and the Low treatment on the third date. The most notable aspect of the interaction is the consistently low densities on the High treatment throughout the season. The significant interaction for the family Laelapidae was erratic and not considered to reflect any trends related to SO_2 fumigation.

Many of the microarthropod groups included in the ANOVA's had significant population changes across time. Most of the groups had seasonal trends like those depicted in Figures 15.5B, 15.6B, and 15.9A and B where the populations were high in the first half of the season and sharply declined during the second half. Because of the sharp population declines late in the season, treatment effects were not observable among many groups. The SO_2 treatments appeared not to cause any major changes in the timing of the late season population declines.

DISCUSSION

Soil microarthropod populations were quite similar in size and structure on the two field sites in 1976 when season mean populations are comparable. Although not tested statistically, there was a substantial difference between the 1975 and 1976 seasons on Site I (Table 15.1).

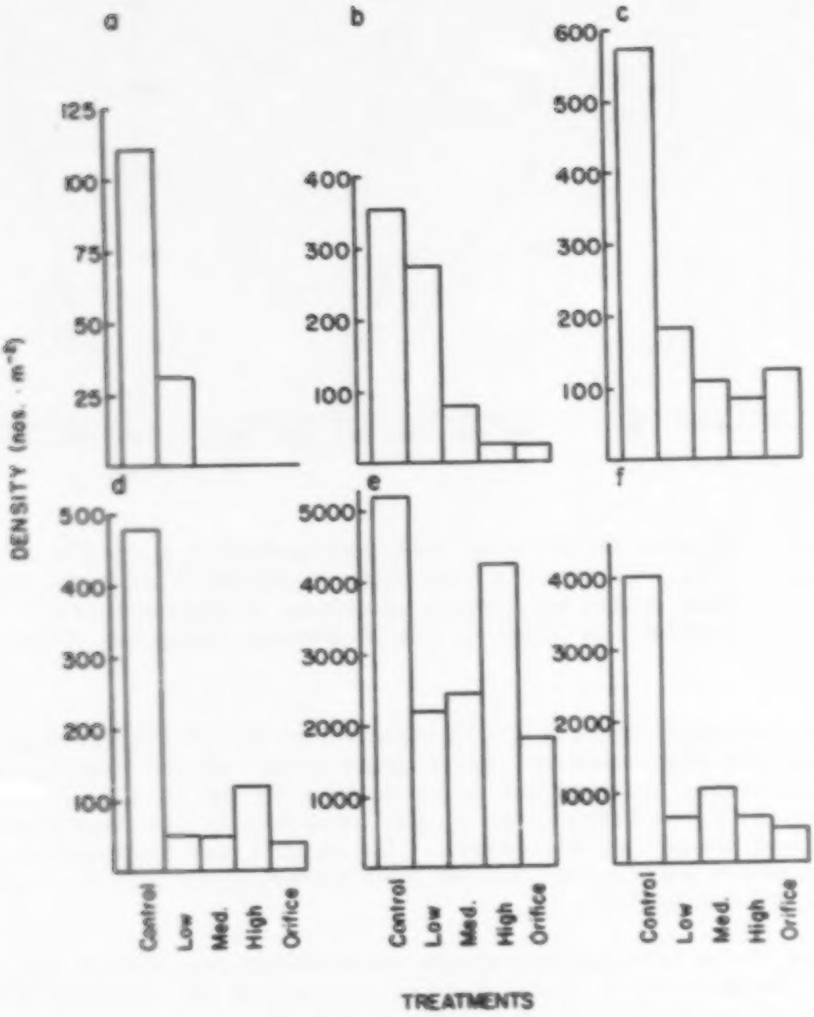

Figure 15.7. Density estimates of five prostigmatid mite families on Site I on 10 August 77 and 8 July 78. A. Pediculochelidae, 1977; B and C. The undescribed endeostigmatid family, 1977 and 1978 respectively; D. Eupodidae, 1978; E. Rhagidiidae, 1978; and F. Tetranychidae, 1978.

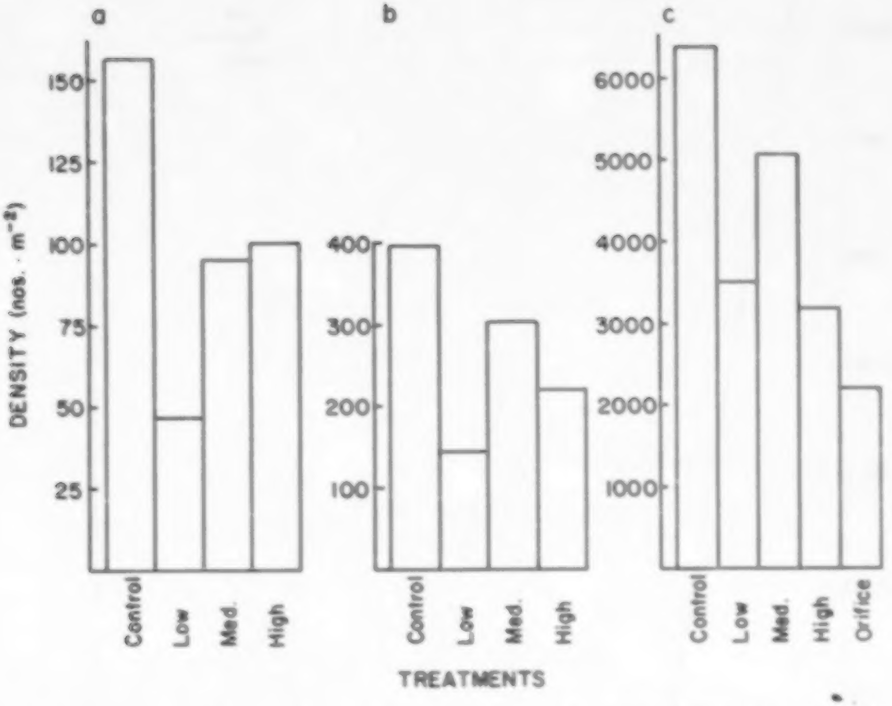

Figure 15.8. Density estimates of three acarine families on Site II. A. Season mean densities for Trombidiidae, 1976; B. Season mean densities for Scutoverticidae, 1976; and C. Densities on 10 August 77 for Nanorchestidae.

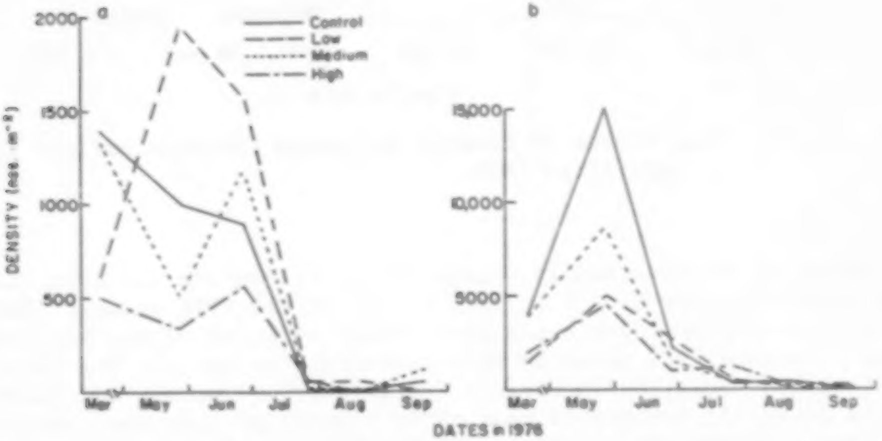

Figure 15.9. Time traces of density estimates for two prostigmatid mite families on Site II in 1976. A. Eupodidae and B. Scutacaridae.

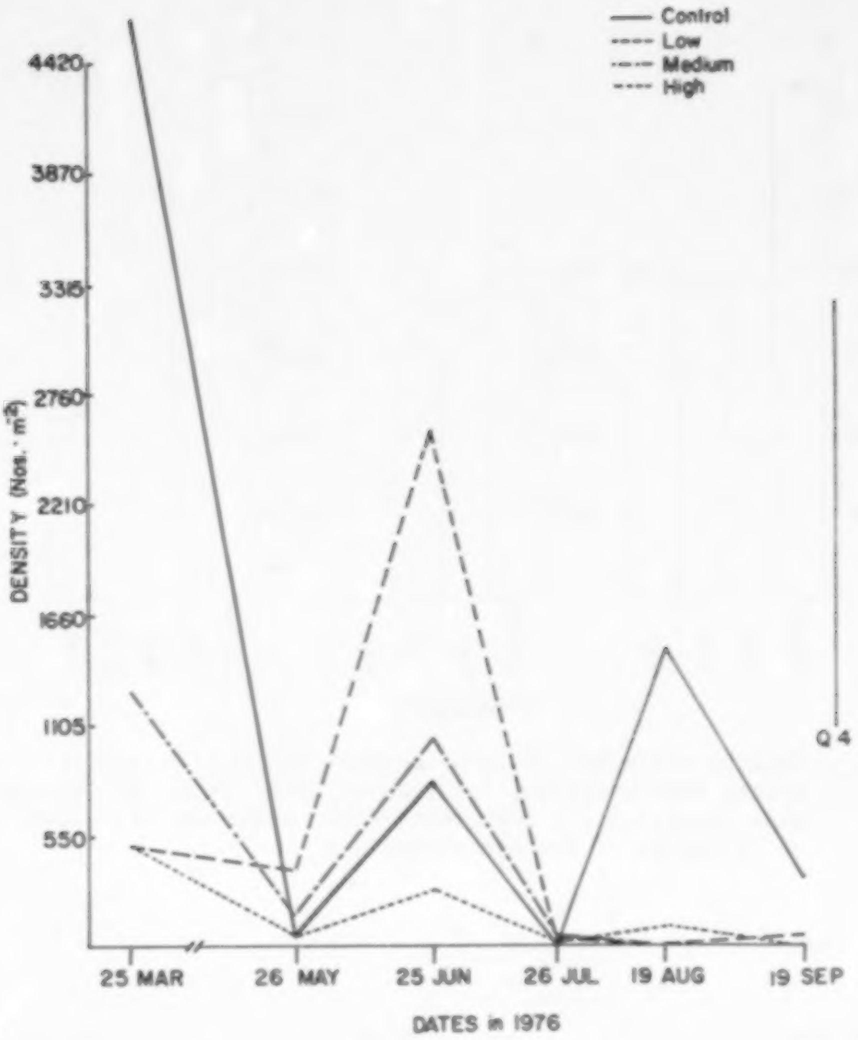

Figure 15.10. Time traces of density estimates for Sminthuridae (Collembola) on ZAPS II in 1976.

Three of the four family categories of Collembola had significantly reduced populations on Site I in part or all of the 1976 season. The fourth family, Onychiuridae, was not significantly affected by the SO_2 treatments possibly because that group is more subsurface in habit. The three affected families, Poduridae, Entomobryidae (including Isotomidae) and Sminthuridae, are all generally surface or near surface dwelling (Leetham, unpublished data from a shortgrass prairie). Since 1975 was the first year of treatment (beginning in June), SO_2 treatment may not have had sufficient time to affect the collembolan populations. This argument seems to hold true for Site II in 1976 where only the Sminthuridae were affected by SO_2 enough to show population reductions. In 1977 and 1978, the single samplings may have been made after the critical moist part of the season when it appears the SO_2 may have its greatest impact. The 1976 date by treatment interactions for the Entomobryiidae and Sminthuridae support this suggestion. Seasonal dynamics of most all the microarthropod groups follow closely the seasonal soil water dynamics - *i.e.*, wet in spring and early summer, drying out in late summer and fall prior to rewetting by fall and winter storms (Dodd *et al.*, 1979).

Although trends of population declines among the soil acarines were numerous, most were not statistically significant because of high sample variability. Where acarine population declines were significant, most occurred during the first half of the season much as among the Collembola. The inconsistencies of apparent population changes among the acarine groups across years and sites adds difficulty in drawing conclusions about the effects of SO_2 fumigation. However, because there are so many trends, we are concluding that SO_2 did have deleterious effects on the soil microarthropods.

The results of this study are not conclusive enough to support a statement of differential sensitivity of the three major trophic classifications used in this study - herbivores, fungivores, and predators. Significant population reductions occurred in representatives of all three. Population changes observed in this study are likely due to direct toxicity of SO_2 or its derivaties on the microarthropods themselves, or a reduction in their food resources through toxicity to prey organisms. Reductions in fungivores is probably due to reductions in food resources since there is ample evidence that SO_2 and its derivatives have significant effects on soil microbial activity (Babich and Stotzky, 1974) which probably reflects reduced microbial populations.

The ecological implications of microarthropod population reductions from SO_2 or other anthropogenic contaminants are largely speculative primarily because the functional importance of these organisms in ecosystem processes is poorly understood. In terms of energy flow as a function of density, biomass, and respiration, the microarthropods are greatly outranked in the soil by microbes and nematodes. However, the importance of the microarthropods may be more along the lines of how they influence the functioning of other groups. For example, the cryptostigmatid mites, which are largely fungivores, can greatly enhance the activities of bacteria and fungi by distributing inoculum (spores, *etc.*) among organic debris (Wallwork, 1970). Parkinson *et al.* (1979) suggest that fungal grazing by Collembola not only can spread fungal spores, but may very likely alter competitive relationships of fungal species complexes in litter and/or soil. This same concept can be projected to nematode-feeding microarthropods.

If direct toxicity of SO_2 is the reason for a decline in the population of one or more microarthropod species, then those same organisms may function in the future as sensitive indicators of changes in the soil-litter system as a result of exposure to anthropogenic SO_2. The full impact of any changes in the litter-soil system as a result of SO_2 exposure can only be evaluated with time so that the long-term effects of small changes such as observed in this study can be related to long-term alterations in ecosystem structure and/or function. Such was not the scope of this study.

CONCLUSIONS

Long-term, low-level SO_2 exposure resulted in significant population reductions among a few of the soil microarthropod groups on the two field sites, although their reductions were not large enough to affect the total microarthropod population estimates or estimates of the dominant group, Acarina. The acarines accounted for over 90 percent of the microarthropods density and 70 percent of the biomass. The most notable treatment effects occurred in the apterygote insect order Collembola where significant population reductions occurred in SO_2 treated plots for season mean collembolan density and the season mean density and biomass of three family groups Poduridae, Entomobryidae (including Isotomidae) and Sminthuridae. These reductions occurred in 1976 on Site I, the second season of SO_2 fumigation. No significant population changes were observed in the first year of fumigation on either Site I or II with the exception of Sminthuridae on some sample dates on Site II in 1976.

Among the Acarina, 11 families were observed to have significantly reduced populations on at least one site during the study. Although the population reductions were statistically significant on only one site-year and/or time-date within a year, trends for these same families and others were observed at other times or dates in the study. High sample variability was the principal reason for failure of many groups to show statistically significant treatment effects.

Sulfur dioxide effects were not restricted to one functional or trophic group. Among those families which had population reductions were representatives of three important trophic groups, *i.e.*, herbivores, predators, and fungivores. Most population reductions observed in this study occurred in the first half of the growing season when soil water conditions were highest. This suggests that the effects of SO_2 on microarthropods are magnified by increased soil moisture, although the mechanism by which SO_2 caused the population changes was not determined. It is possible that both direct toxicity and toxicity to food resources could have been involved in the population changes observed.

REFERENCES

Abrahamsen, G., J. Hovland, and S. Hågvar. 1978. Effects of Artificial Acid Rain and Liming on Soil Organisms and the Decomposition of Organic Matter. SNSF-contribution FA 28/78. 23 pp.

Bååth, E., B. Berg, U. Lohm, B. Lundgren, H. Lundkvist, T. Rosswall, B. Söderstrom, and A. Wiren. 1980. Effects of Experimental Acidification and Liming on Soil Organisms and Decomposition in a Scots Pine Forest. Pedobiologia. (In Press)

Babich, H. and G. Stotzky. 1974. Air Pollution and Microbial Ecology. Crit. Rev. Environ. Control, 4:353-421.

Dodd, J. L., J. W. Leetham, T. J. McNary, W. K. Lauenroth, and G. L. Thor. 1979. Baseline Characteristics of Producer and Invertebrate Populations and Certain Abiotic Parameters in the Colstrip Vicinity, pp. 53-106. In: E. M. Preston and T. L. Gullett (eds.) The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report. EPA-600/3-79-044. U. S. Environmental Protection Agency. Corvallis, Oregon.

Lebrun, P., G. Wauthy, C. Leblanc, and M. Goossens. 1977. Ecologic Test of the Tolerance to SO_2 Toxicity in the Oribatid Mite *Humerobates rostrolamellatus* (Grandjean, 1936) (Acari: Oribatei). Annales Soc. R., Zool., Belg., 106:193.

Lebrun, P., J. M. Jacques, M. Goossens and G. Wauthy. 1978. The Effect of Interaction Between the Concentration of SO_2 and the Relative Humidity of Air on the Survival of the Bark-living Bioindicator Mite *Humerobates rostrolamellatus*. Water, Air, and Soil Pollut., 10:269-275.

Leetham, J. W., T. J. McNary, J. L. Dodd, and W. K. Lauenroth. 1979. Response of Field Populations of Tardigrada to Various Levels of Chronic, Low-level Sulfur Dioxide Exposure. Proceedings of VII International Colloquim of Soil Zoology.

Merchant, V. A. and D. A. Crossley. 1970. An Inexpensive High Efficiency Tullgren Extractor for Soil Microarthropods. J. Ga. Entomol. Soc., 5:83-87.

Parkinson, D., S. Visser, and J. B. Whittaker. 1979. Effects of Collembolan Grazing on Fungal Colonization of Leaf Litter. Soil Biol. Biochem., 11:529-535.

Wallwork, J. A. 1970. Ecology of soil animals. McGraw-Hill Publishing Company Limited, Maidenhead, Berkshire, England. 283 pp.

SECTION 16

ARTHROPOD POPULATION RESPONSES TO THREE LEVELS OF CHRONIC SULFUR DIOXIDE EXPOSURE IN A NORTHERN MIXED-GRASS ECOSYSTEM

II ABOVEGROUND ARTHROPODS

J. W. Leetham, J. L. Dodd, R. D. Deblinger, and W. K. Lauenroth

ABSTRACT

The effects of continuous, season-long exposure of aboveground arthropods to various levels of SO_2 were investigated under field conditions in 1975 and 1976 in southeastern Montana. Three field plots (0.52 ha each) on each of two grassland community sites were fumigated with SO_2 on the ZAPS sites (Section 1). Similar sized Control plots were included in each site. Periodic samplings were made throughout the 1975 and 1976 growing seasons. Aboveground arthropods were sampled by dropping a 0.5 m circular cage over predetermined (but randomly chosen) sample locations and retrieving the arthropods by vacuum. Berlese funnel extraction and hand sorting were used to separate arthropods from debris. Biomass and/or density reductions occurred in a number of insect and acarine groups on one or both sites. The groups included Acarina, Diplura, Collembola (Poduridae), Hemiptera (Pentatomidae), Homoptera (Cicadellidae), Thysanoptera (Thripidae), Coleoptera (total) Staphylinidae and Curculionidae, Diptera (Muscidae and Ceratopogonidae). These taxonomic groups were considered to represent two possible classifications of organisms showing population reductions. One group included arthropods which were relatively immobile and strongly associated with the soil surface litter. A second group included more mobile, flying insects. It is suggested that reductions in populations in the former group most likely involve toxic effects of SO_2 or its derivatives directly to the organisms or their food resources. The mobility of the latter group introduces the possibility of behavioral avoidance of the relatively small experimental plots. Evidence to support these hypotheses is given.

INTRODUCTION

The origin and importance of this study as a portion of a large, interdisciplinary research project concerned with coal-fired power plant emissions on northern mixed-grass prairie were discussed in Leetham *et al.* (submitted b). This paper will concern responses of aboveground arthropods to long-term, low-level sulfur dioxide exposure under field conditions.

Three aspects of this study make it unique among studies of air pollutants and arthropods: 1) a system level orientation to responses of arthropods in a native prairie to exposure to a major component of coal-fired plant emissions; 2) the study area was considered pristine (*i.e.*, no previous history of air pollution exposure) prior to experimental fumigation with SO_2; and 3) the study was conducted under field conditions. Of the few published studies concerning insects and air pollutants, most are either *a posteriori* in that they were conducted sometime after the experimental areas were exposed to pollution, or the studies were conducted under highly controlled laboratory conditions. For example, Freitag *et al.* (1973) studied carabid beetle populations near a Kraft mill in Thunder Bay, Ontario, some years after the mill had been operating, and found lower beetle populations near the mill as compared to farther downwind. Hillman and Benton (1972) concluded that SO_2 exposure from a coal-fired power plant in central Pennsylvania was responsible for reduced populations of social bees and parasitic wasps, and the reduced wasp population was, in turn, responsible for increased aphid populations. They followed-up the field study by fumigating honey bee colonies with SO_2 at various controlled levels between 0 and 500 pphm and found an inverse relationship of brood-rearing and pollen collection with SO_2 concentration. Ginevan and Lane (1978), using *Drosophila melanogaster* and controlled laboratory conditions found long-term, low-level SO_2 exposure (70 and 40 pphm) caused significant increases in developmental time and decreases in survival.

MATERIALS AND METHODS

The study area in southeastern Montana and design of the field experimental plots were described in Leetham *et al.* (submitted b). Aboveground arthropods were sampled on Site I 6 times in each 1975 and 1976 growing season while Site II was sampled 6 times in the 1976 season only. Samplings for a given year were spaced at approximately 3 week intervals throughout the growing season (April - September).

For this study, "aboveground arthropods" were defined as those arthropods occurring in or above the soil litter and aerial vegetation. The arthropods were sampled by dropping a circular cage over predetermined, but randomly chosen, sample locations and vacuuming out the contents, including litter and vegetation. The cage covered 0.5 m^2 and was dropped from a cart-mounted 18 foot (5.5 m) boom. Cage contents were vacuumed in two stages: 1) a light vacuuming of cage walls and aerial vegetation for active arthropods, and 2) clipping and bagging of all vegetation followed by hand vacuuming to retrieve remaining litter and plant refuse. The first stage material was frozen then hand sorted for arthropods while the second stage was subjected to Berlese funnel extraction to retrieve the arthropods. The two stage process was found to have better efficiency of retrieval than other techniques such as hand sorting and mechanical flotation. All the arthropods were preserved in 70 percent ethanol and later counted and identified. Five samples per replicate (10 per treatment) were taken on all dates, the sample locations being chosen by use of a random numbers table. Specific details of the collecting and extracting equipment are given by Leetham (1975).

The arthropods were identified as far as possible which often was only to family. When possible, voucher specimens were sent to recognized specialists for verification. Representatives of all taxa were dried at 65°C for 24 hours and weighed for dry weight biomass. A split plot analysis of variance was performed on the data to test for treatment and date-within-season effects. Because Site II was not fumigated until 1976, we concluded it would be illogical to include "sites" and "years" in the ANOVA design. The two sites were not comparable in 1976 because of differences in fumigation history, and first-year fumigation on Site I was not comparable to first-year fumigation on Site II because of differences in growing seasons. Because of high sample variability, the ANOVA was run at the order and family level on selected groups based on whether or not there appeared to be population changes in the treated plots (judged on season mean density and/or biomass summaries). Individual analyses were performed on both density and biomass data of each group because either or both parameters can be used to measure population changes in a given family or order. It should be stated at this point that although the traditional significance level of $P = 0.05$ was used as a guideline in judging population changes as significant, probability levels of $0.05 < p < 0.10$ were accepted for one or more site-years for a given group if that group showed similar trends (whether significant or not) in other site-years. Tukey's Q procedure was used to calculate least significant ranges which were used to compare treatments or dates when the ANOVA results indicated significant population changes. In cases where both a main effect of treatment or date and a date-by-treatment interaction were significant, the interaction was given presidence over the main effect. In most cases where this situation occurred, it was because the particular group was abundant only for a portion of the season and significant treatment differences occurred at that time. During the remainder of the season, the group did not occur or did so in such low densities that treatment differences were not measurable.

RESULTS

The list of identified arthropods collected during this portion of the study includes 15 orders and 60 families of insects, five families of spiders (Araneida), five families of mites (Acarina), and centipedes (Geophilomorpha). A general overview of the aboveground arthropod community structure on Sites I and II is provided in Tables 16.1 and 16.2. Presented are density and biomass estimates for the major orders and families. The major order on both sites was Coleoptera with Hymenoptera and Homoptera following in that order (based on biomass). The Curculionidae (weevils) was the major coleopteran family, Formicidae (ants) the major hymenopteran family and the Cicadellidae (leaf hoppers) the major homopteran family. A complete list of all families, genera and species is far too voluminous to include here.

TABLE 16.1. GENERAL ABOVEGROUND ARTHROPOD COMMUNITY STRUCTURE OF SITE I IN 1976*

Order	Family	Density (Nos · m^{-2})				Biomass (mg · m^{-2})			
		Cont.	Low	Med.	High	Cont.	Low	Med.	High
Araneida		2.5	3.3	2.9	3.6	1.6	1.3	6.3	5.0
"	Lycosidae	<0.1	0	0.2	0.3	0.2	0	5.0	3.5
Coleoptera		55.8	44.3	42.3	41.8	64.4	56.8	53.6	62.6
"	Carabidae	5.2	4.0	3.5	3.8	7.7	5.5	7.5	7.3
"	Curculionidae	10.1	10.6	8.2	10.8	28.5	30.0	23.9	29.5
"	Elateridae	<0.1	0.2	<0.1	0.2	<0.1	1.5	0.1	2.7
"	Staphylinidae	6.9	4.5	4.6	5.0	3.8	2.3	1.7	2.9
"	Tenebrionidae	0.3	0.7	0.7	0.3	1.1	1.7	2.3	1.3
"	Chrysomelidae	10.2	7.9	7.6	6.0	12.0	8.8	9.6	11.0
Collembola		173.6	6.1	17.1	91.1	3.5	0.1	0.3	1.8
"	Poduridae	173.6	6.1	17.1	91.1	3.5	0.1	0.3	1.8
Hemiptera		11.9	14.3	12.7	18.3	9.7	8.8	6.5	12.1
"	Cydnidae	0.4	0.2	<0.1	1.0	1.6	0.8	0.1	3.9
"	Lygaeidae	8.7	10.9	9.9	13.8	3.2	3.2	2.8	3.9
"	Miridae	1.3	1.3	1.8	2.6	1.2	1.3	1.5	2.1
"	Nabidae	0.6	0.9	0.6	0.4	1.3	1.9	1.3	0.8
"	Scutelleridae	0.1	0.1	<0.1	0.1	1.2	0.7	0.3	0.9
Homoptera		50.2	21.2	19.9	26.6	13.8	9.9	7.7	9.6
"	Cercopidae	0.1	0.2	0.1	0.3	0.7	0.8	0.5	1.3
"	Cicadellidae	12.6	14.0	8.9	6.7	9.5	8.1	5.7	6.3
"	Pseudococcidae	14.8	4.4	7.3	11.9	1.3	0.4	0.7	1.1
Hymenoptera		22.5	27.7	34.3	26.7	15.1	14.0	18.3	15.1
"	Formicidae	20.6	25.4	33.0	24.2	14.4	12.9	17.4	12.9
Lepidoptera		1.1	0.5	0.9	0.7	5.7	1.3	2.0	5.7
"	Noctuidae	0.1	0	0	0.1	3.4	0	0	4.6
Orthoptera		0.4	0.4	0.6	0.6	2.2	3.8	8.2	11.4
"	Acrididae	0.4	0.3	0.6	0.5	2.2	1.5	7.4	11.4
Thysanoptera		83.4	27.8	22.5	35.6	3.3	1.0	0.9	1.4
"	Thripidae	83.4	27.8	22.5	35.6	3.3	1.0	0.9	1.4
Total Arthropods		420.4	164.1	166.3	255.3	121.1	98.4	105.6	125.8

* Data are season means. The list includes groups where 1.0 mg · m^{-2} occurred on one or more treatments.

The three site-year combinations present difficulties in analyzing and interpreting the resulting data. As mentioned previously, first year exposure on Sites I and II are not directly comparable because they occurred in separate seasons and the only second-year data collected were from Site I in 1976. Because of these problems, potential inconsistencies between site-years in measured arthropod responses to SO_2 fumigation could very well be expected and, in fact, did occur. Tables 16.3 and 16.4 list those orders and families of arthropods which had trends of population density and/or biomass changes with SO_2 exposure, based on season mean density and biomass estimates. Individual ANOVA's were performed for each of the groups listed. The results will be discussed here by site-year.

1975 - Site I

Total coleopteran density was reduced for part of the 1975 season resulting in a significant treatment-by-date interaction (Figure 16.1A). This is quite probably due to a similar trend for the density and biomass of

TABLE 16.2. GENERAL ABOVEGROUND ARTHROPOD COMMUNITY STRUCTURE OF SITE II IN 1976*

Order	Family	Density ($Nos \cdot m^{-2}$)				Biomass ($mg \cdot m^{-2}$)			
		Cont.	Low	Med.	High	Cont.	Low	Med.	High
Araneida		2.5	1.4	1.7	1.9	3.0	5.1	4.4	6.6
"	Lycosidae	0.7	0.1	0.1	0.2	1.6	4.3	3.1	5.3
Coleoptera		49.4	53.6	41.3	24.4	80.3	90.3	77.1	40.9
"	Carabidae	4.3	6.1	4.2	2.4	7.6	6.9	7.4	2.2
"	Chrysomelidae	8.5	4.6	5.3	3.2	14.8	7.2	7.7	4.4
"	Curculionidae	15.6	17.8	14.8	9.8	47.5	53.2	42.0	28.5
"	Elateridae	0.9	1.8	0.5	0	2.2	6.0	1.9	0
"	Orthoperidae	4.6	5.5	2.9	2.1	1.0	1.2	0.6	0.5
"	Staphylinidae	5.9	5.0	3.0	3.1	3.1	2.5	1.4	0.9
"	Tenebrionidae	0.7	3.2	4.0	1.0	2.6	11.5	14.6	3.2
Hemiptera		10.3	14.0	9.7	9.0	10.3	9.3	6.4	7.2
"	Lygaeidae	7.7	8.8	7.8	5.9	2.8	2.8	2.7	1.7
"	Cydnidae	0.9	0.2	0.1	<0.1	3.6	0.7	0.4	0.1
Homoptera		19.5	24.4	14.9	13.4	5.5	7.6	5.2	6.1
"	Cicadellidae	4.2	4.2	2.3	3.6	3.6	4.7	3.0	4.4
"	Pseudococcidae	11.9	11.6	11.0	9.1	1.1	1.5	1.0	0.8
Hymenoptera		20.8	36.6	32.8	29.7	9.9	16.6	14.6	13.2
"	Formicidae	19.5	35.7	32.4	29.2	9.5	16.1	14.5	12.8
Lepidoptera		1.1	1.5	1.2	1.0	2.8	2.7	1.0	4.0
"	Noctuidae	<0.1	<0.1	0	<0.1	1.1	1.1	0	2.3
Orthoptera		0.5	0.7	0.8	0.9	1.9	3.6	22.3	19.8
"	Acrididae	0.5	0.7	0.8	0.8	1.9	3.6	22.3	17.4
Thysanoptera	Thripidae	31.4	22.2	23.2	10.3	1.1	0.9	0.9	0.4
Total Arthropods		157.9	170.7	141.0	100.5	117.2	138.9	133.7	99.8

* Data are season means. The list includes groups where 1.0 $mg \cdot m^{-2}$ occurred on one or more treatments.

the major coleopteran family Curculionidae (Figure 16.1B). Nine species of curculionid beetles were collected on Site I in 1975 and 1976, two of which account for over 90 percent of the total density and biomass for the family - *Hyperodes grypidioides* Dietz and *H. vitticollis* (Kirby). The *H. grypidioides* was collected only in the spring and fall (probably overwintering as adults) and *H. vitticollis* was collected only during the mid part of the growing season.

Significant treatment effects occurred for both density and biomass for the order Homoptera and the family Pseudococcidae. However, the significant reductions occurred in the Low and Medium treatments while the Control and High plots were not significantly different from each other. The date by treatment interactions reveal high pseudococcid (and total Homoptera) populations on the Control in spring and similarly high populations on the High treatment in the fall (data are not presented). Because of these erratic trends, the population differences among the treatments are not considered as the result of SO_2 fumigation but chance variation among the field plots.

Although both density and biomass of the acarine family Parasitidae were reduced in all treated plots, none were significant because of high sample variability.

TABLE 16.3. ANOVA RESULTS FOR ARTHROPOD BIOMASS ON THREE SITE-YEARS. NUMBERS ARE THE SIGNIFICANCE LEVELS FOR EACH TEST.

Group		Site I - 1975		Site I - 1976		Site II - 1976	
		Treat*	TxD†	Treat	TxD	Treat	TxD
Geophilomorpha				-	-	-	.060
Araneida	Dictinidae					.008	.000
Acarina (total)						-	-
"	Tydeidae					.030	-
"	Oribatulidae					-	.032
"	Parasitidae	-‡	-				
Collembola (total)				-	.007		
"	Poduridae			-	.007		
"	Sminthuridae					-	-
Diplura						-	-
Hemiptera	Pentatomidae			.009	-		
Homoptera (total)		.012	-	.003	.000	-	-
"	Cicadellidae			.055	-		
"	Pseudococcidae	.013	.000	.030	.001		
"	Psyllidae			-	.000		
"	Aphididae					-	-
Thysanoptera	Thripidae			.038	.007	.013	.071
Coleoptera (total)		-	-	-	.011	.029	-
"	Anthicidae	-	-				
"	Curculionidae	-	.075				
"	Leptodiridae					-	-
"	Staphylinidae					.047	-
Diptera (total)				.089	.002		
"	Ceratopogonidae			.007	.000		
"	Chloropidae			-	-		
"	Muscidae			.049	.058		
"	Sphaeroceridae			-	-		
Hymenoptera	Diapriidae					-	.060

* Treatment main effect.

† Treatment by date interaction.

‡ Indicates the group showed a trend of season mean population reduction with SO_2 exposure, but reductions were not significant at $P \leq 0.10$. Blank spaces indicate no trends and hence not tested statistically.

TABLE 16.4. ANOVA RESULTS FOR ARTHROPOD DENSITIES ON THREE SITE-YEARS. NUMBERS ARE THE SIGNIFICANCE LEVELS FOR EACH TEST.

Group		Site I - 1975		Site I - 1976		Site II - 1976	
		Treat*	TxD†	Treat	TxD	Treat	TxD
Geophilomorpha				-	-	-	-
Araneida	Dictinidae					.008	.000
Acarina (total)						.084	-
"	Tydeidae					.030	-
"	Oribatulidae					-	.032
"	Parasitidae	-‡	-				
Collembola (total)				-	.007		
"	Poduridae			-	.007		
"	Sminthuridae					-	-
Diplura						.048	-
Hemiptera	Pentatomidae			-	-		
Homoptera (total)		.005	.000	.020	.000	-	-
"	Cicadellidae			-	-		
"	Pseudococcidae	.012	.000	.032	.001		
"	Psyllidae			.045	.000		
"	Aphididae					-	-
Thysanoptera	Thripidae			.033	.004	.025	.026
Coleoptera (total)		-	.020	-	.006	.075	.043
"	Anthicidae	-	-				
"	Curculionidae	-	.008				
"	Leptodiridae					-	-
"	Staphylinidae					-	-
Diptera (total)				-	-		
"	Ceratopogonidae			.007	.000		
"	Chloropidae			-	-		
"	Muscidae			.048	.056		
"	Sphaeroceridae			-	-		
Hymenoptera	Diapriidae					-	.003

* Treatment main effect.

† Treatment by date interaction.

‡ Indicates the group showed a trend of season mean population reduction with SO_2 exposure, but reductions were not significant at $P \leq 0.10$. Blank spaces indicate no trends and hence not tested statistically.

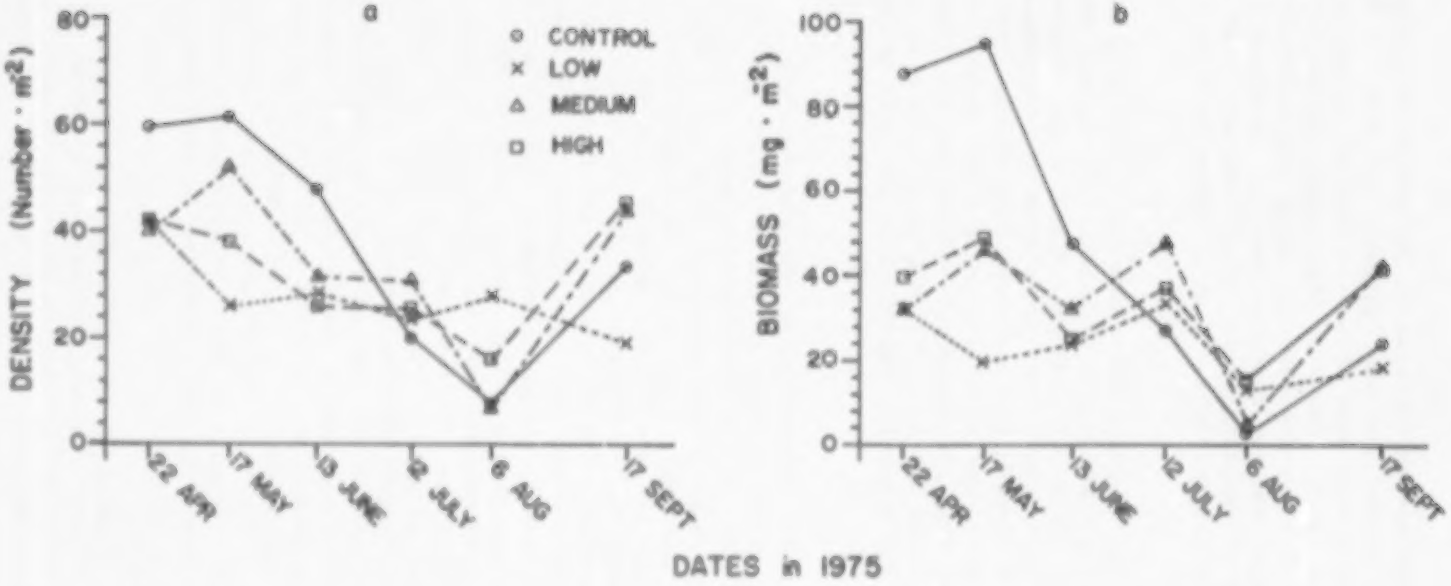

Figure 16.1. Time traces of two insect groups on Site I in 1975. (A) Coleoptera (Total) density. (B) Curculionidae biomass.

1976 - Site I

As expected, there was an increase in the number of groups showing apparent responses to second year SO_2 fumigation on Site I. Fifteen groups had reductions in season mean density and/or biomass in one or more fumigated plots (Tables 16.3 and 16.4). However, high sample variability again was responsible for some of the reductions not being statistically significant, including Geophilomorpha and two dipteran families Chloropidae and Sphaeroceridae. Many of the significant reductions were confused by significant treatment-by-date interactions. Each of these cases will be discussed separately.

The collembolan family Poduridae which was represented in these data by only one species, *Hyogastrura armata* (Nicolet), had reduced densities and biomass on all treated plots but none were significant. However, the treatment-by-date interactions were significant and a plot of the biomass data (Figure 16.2A) shows *H. armata* was collected only on the first two sample dates at which time there were significantly smaller populations on the Low and Medium plots. The High treatment was reduced but not significantly. Certainly *H. armata* is not the only collembolan occurring on the field plots, but it is the major species occurring on the soil surface in late winter and early spring. It often is found in large numbers on or near water puddles.

One hemipteran family, Pentatomidae, represented largely by *Neotiglossa sulcifrons* Stal. late in the growing season, had significantly reduced season mean biomass on the Medium and High treatment plots (Figure 16.3A). The order Homoptera had significant treatment effects for both density and biomass, however, in both cases there also were significant interactions. A plot of the density data (Figure 16.2B), which is similar to that for biomass, shows a significant late season increase in the populations on the Control plot while similar increases did not occur in any treated plot. The families Cicadellidae and Pseudococcidae made up a majority of the Homoptera. The cicadellids had significantly reduced biomass on the Medium and High treatments (Figure 16.3B). Numerous genera and species of cicadellids were collected thoughout the season with various seasonal abundance patterns. The population reductions appeared not to be the result of one major species being affected but a general reduction of most of the dominant species. Only one species of pseudococcid was collected (*Distichlicoccus* sp.) and it occurred throughout the season. It had significantly reduced densities and biomass on the low and Medium plots on the first sample date as determined from the interaction. Trends were quite erratic and possibly not a reflection of SO_2 fumigation. The family Psyllidae had reduced biomass and densities on all treated plots late in the season, but the only significant treatment differences occurred on the fifth sample date (Figure 16.2C). No representatives of the only genus collected, *Craspedolepta*, were collected in any treated plot after the third sampling.

Both density and biomass of Thripidae (Thysanoptera) were reduced late in the season. Significant treatment differences occurred only on the fifth date (Figure 16.2D), where all treatments were significantly lower than the Control, however a trend of reduced populations in all treatments occurred across the last four sample dates. At least eight species of thrips, all of

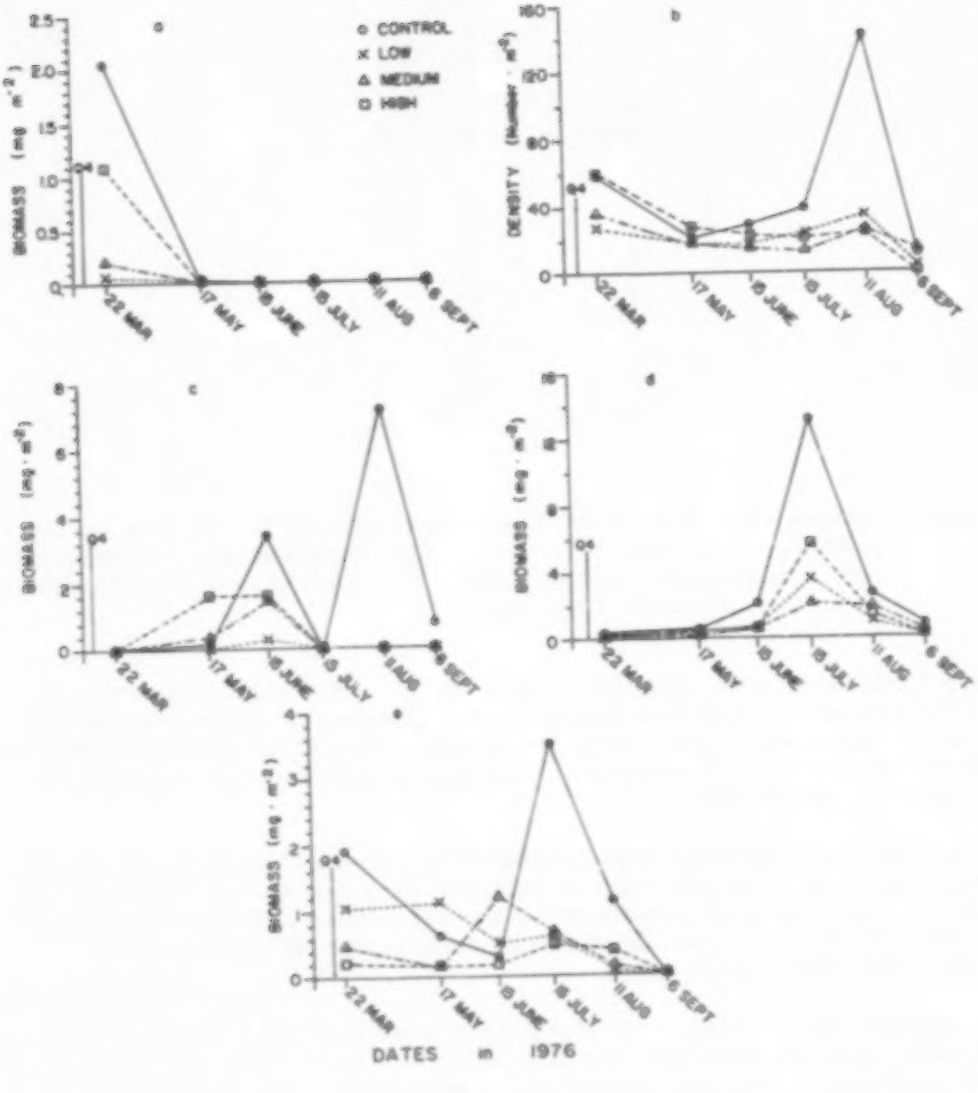

Figure 16.2. Time traces of five insect groups on Site I in 1976. (A) Poduridae (Collembola), (B) Homoptera (Total), (C) Psyllidae (Homoptera), (D) Thripidae (Thysanoptera), (E) Diptera (Total).

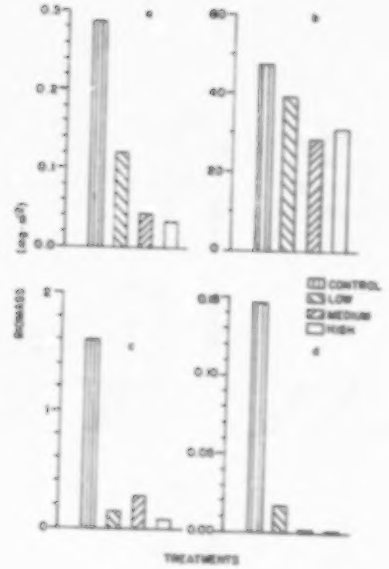

Figure 16.3. Season mean biomass of four insect groups on the four treatment plots of Site I in 1976. (A) Pentatomidae (Hemiptera), (B) Cicadellidae (Homoptera), (C) Ceratopogonidae (Diptera), and (D) Muscidae (Diptera).

the family Thripidae, were collected, only two of which were identified to genus - *Chirothrips* sp. and *Frankliniella* sp. Neither of these two species were of major importance among those collected. Four other species comprised the majority of the thrip biomass and they were generally most abundant in the last half of the season.

Significant treatment-by-date interactions for total Coleoptera density and biomass resulted when the Control differed from the Medium treatment on the third date and the High treatment on the fifth date (data not presented). No other significant treatment differences occurred which suggests the differences were chance happenings.

The significant treatment-by-date interaction for total dipteran biomass resulted when the High treatment was significantly lower than the Control on the first sample date and all treated plots were lower than the Control on the fourth date (Figure 16.2E). As with the total Coleoptera, there are no consistent trends across all dates, so it becomes questionable if the two significant points represent treatment differences. Both dipteran families with significant population reductions, Muscidae and Ceratopogonidae, were collected in the larval stages from the soil surface litter. *Fannia* sp. (Muscidae) was collected on only two sample dates (17 May and 12 July) and the ceratopogonid (species unidentified) were collected from all treatments on 22 March 1976 but only from the Control plot on 17 May and was not collected from any of the plots during the remainder of the season (Figures 16.3C and 16.3D). The significant interactions were the result of the temporary occurrence on the plots and not from erratic seasonal trends.

1976 - Site II

First year fumigation on Site II resulted in more groups showing population changes than occurred during the first year on Site I. However, again because of high sample variability some of the changes were not significant including Sminthuridae (Collembola), Homoptera (total), and Leptodiridae (Coleoptera). Each of these groups showed trends of density and/or biomass reductions in the treated plots, based on season means.

A plot of the treatment-by-date interaction for centipedes (Geophilomorpha) shows erratic collections in the first half of the season with no collections thereafter (Figure 16.4A). The most consistent characteristic is that no representatives were taken in the High treatment plot and very few in the edium treatment. However, considering the reductions as being the result of SO_2 fumigation is tenuous. One family of spiders (Dictynidae), represented by *Dictyna consulta* Gertch and Ivie and *D. terrestris* Emerton had significantly reduced density and biomass in the Medium and High treatment plots on the third sample date and in all treated plots on the fifth sample date (Figure 16.4B). Total acarine density was reduced on all treated plots although the significance level was only $P = 0.084$ (Figure 16.5A). The density and biomass of the acarine family Tydeidae was significantly reduced on all treated plots (Figure 16.5B). A plot of the date by treatment interaction for Oribatulidae biomass shows a significant reduction on the fifth sampling when populations were at their highest (Figure 16.4C). The density of Diplura was reduced on all treated plots, however, the reduced biomass was not significant (Figure 16.5C).

Both density and biomass of Thripidae (Thysanoptera) were significantly reduced on the High treatment plot (Figure 16.5D) although the significant interaction shows the reduction occurred late in the growing season much the same as on Site I. Total beetle (Coleoptera) biomass was significantly reduced on the High treatment plot (Figure 16.5E) while a significant interaction occurred for density. A plot of the interaction (Figure 16.4D) shows the High treatment to be consistently lower than the Control, Low, and Medium plots, but that significant differences occurred on the third, fifth, and sixth dates. Only one coleopteran family, Staphylinidae, had a significant biomass reduction, where both the Medium and High treatments were reduced from the Control (Figure 16.5F). Three species made up the majority of the Staphylinidae - *Philonthus* sp., *Aleochara* sp., and *Tachyporus* sp. The *Philonthus* was collected throughout the season while the *Aleochara* sp. and *Tachyporus* were collected almost exclusively in the first three samplings. At least six other species were collected but only rarely. The reduction in Staphylinidae was due to reductions in all three of the principal species. One other coleopteran family, Curculionidae, showed substantially reduced biomass on the High treatment, but the data were untested.

One parasitic hymenopteran family, Diapriidae, showed significant interactions for both density and biomass, particularly density (Figure 16.4E).

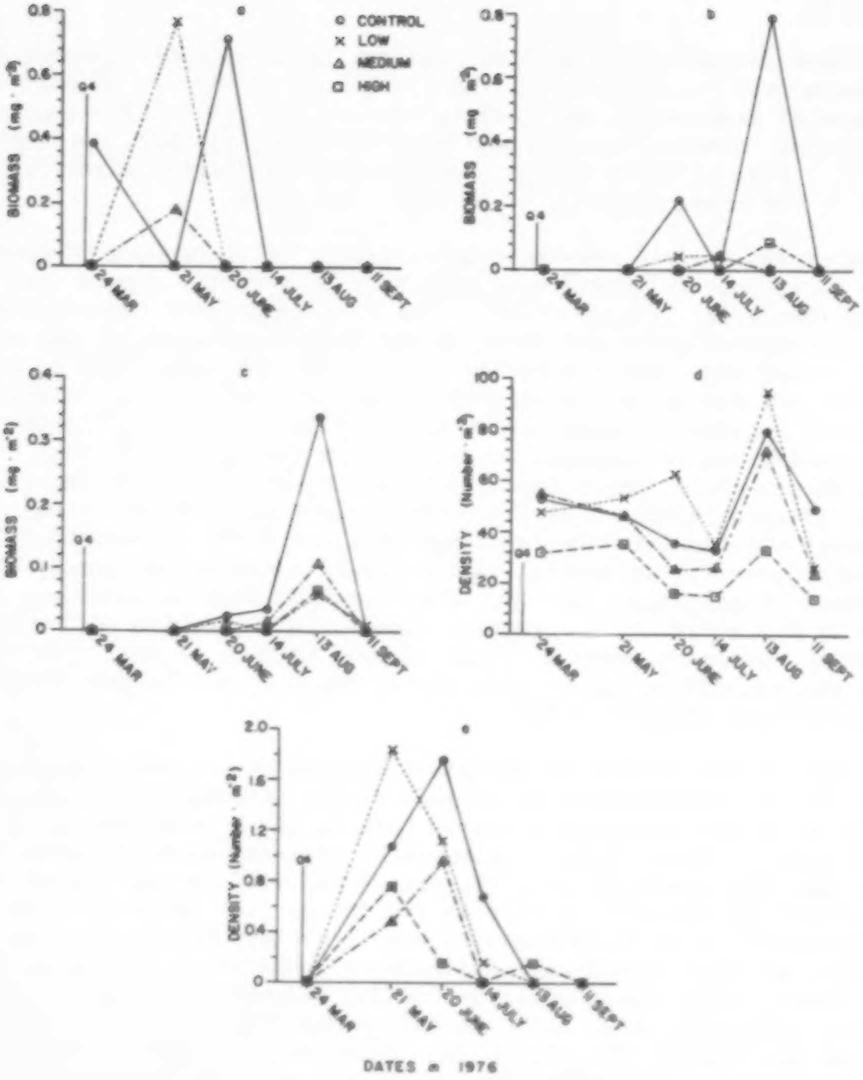

Figure 16.4. Time traces of five arthropod groups on four treatment plots of Site II in 1976. (A) Geophilomorpha, (B) Dictynidae (Araneida), (C) Oribatulidae (Acarina), (D) Coleoptera (Total), (E) Diapriidae (Hymenoptera).

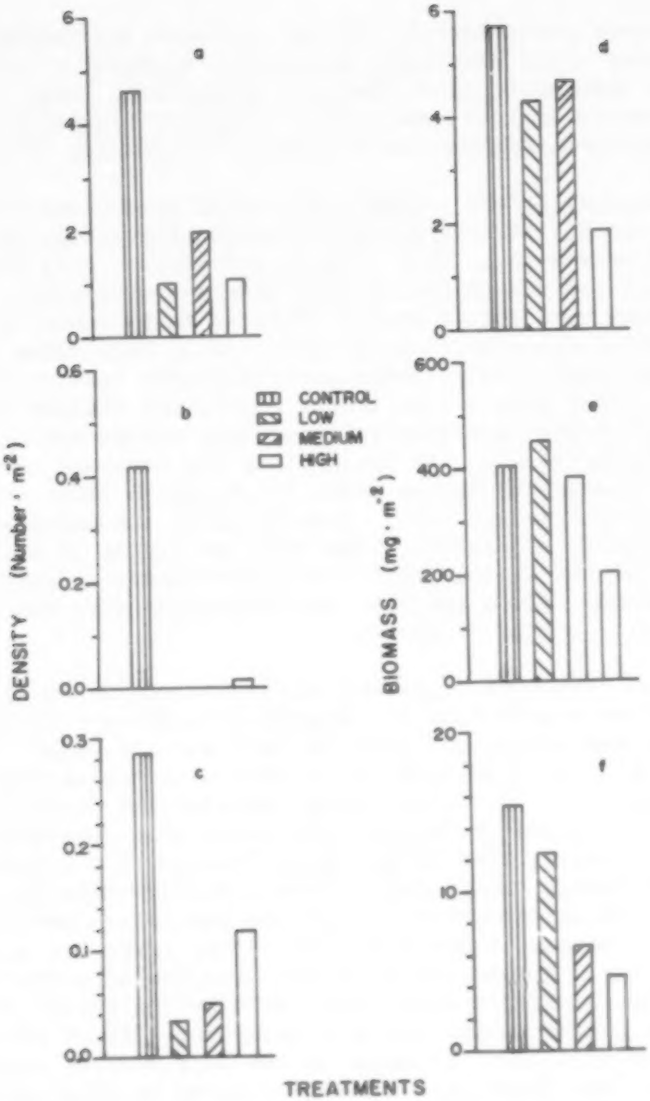

Figure 16.5. Season mean density or biomass of six arthropod groups on four treatment plots of Site II in 1976. (A) Acarina, (B) Tydeidae (Acarina), (C) Diplura, (D) Thripidae (Thysanoptera), (E) Coleoptera (Total), (F) Staphylinidae (Coleoptera).

The reduction occurred early in the season when they were most abundant. No species identifications were made.

DISCUSSION

All arthropod population changes noted here as associated with SO_2 fumigation have been reductions in density and/or biomass. No significant population increases were observed for any group in any season although increases certainly could have been expected as a result of upsetting predator-prey balances or changes in plant host susceptability. Both concepts have been argued as causes of increased insect damage to plants in areas of high air pollutant exposure (Hillman and Benton, 1972; Heagle, 1973).

Although numerous groups showed significant population reductions associated with the SO_2 fumigation in the three site-years, we are concerned over the lack of consistency between sites and years. Only three groups had significant population reductions in more than one site year. Total homopteran density and biomass were significantly affected in 1975 and 1976 on Site I; the family Thripidae was significantly affected on both sites in 1976; and total coleopteran density and biomass were variously reduced in all three site-years. All other groups that showed population changes did so in only one site-year. As stated previously, these inconsistencies were not completely unexpected because of the way the field study was designed and implemented, *i.e.*, first year fumigation on the two sites being in different seasons and only one set of second season data. Despite these inconsistencies, there are enough significant population reductions and trends of such to lead to the conclusion that SO_2 fumigation did have detrimental effects on many aboveground arthropod groups and that the reductions were not chance occurrences among a large number of groups.

The treatment responses of arthropods to long-term low-level SO_2 exposure observed here fall into two general categories - 1) those arthropods (or stages) which are associated with the soil surface litter and relatively non-mobile, and 2) those arthropods associated with aerial vegetation and generally quite mobile. The former group includes the Geophilomorpha, Araneida, Diplura, Acarina, Poduridae, Pseudococcidae, Muscidae (larvae) and Ceratopogonidae (larvae). The latter group includes the Cicadellidae, Thripidae, Staphylinidae, and Curculionidae. Direct toxicity or toxicity to food resources would conveniently explain the population reductions in the first group simply because those organisms cannot leave the plots for mere avoidance of the SO_2. A possible exception here would be the two dipteran families where the population reductions could be the result of avoidance of the treated plots by the adults during oviposition. Since all of the members of the first group were found to occur in the soil surface litter it is quite possible to link their population reductions to other determined effects of SO_2 on the litter, *i.e.*, reduction of decomposition rates of plant litter as a consequence of reduced microbial activity (Leetham *et al.*, submitted a; Dodd and Lauenroth, 1980). Decreased microbial activity may reflect decreased available microbial food reserves for arthropod groups listed, most of which are considered to be utilizing these resources wholly or partially. Certainly direct toxicity is a real possibility for explaining population decreases in all the arthropods since other studies have shown SO_2 can be toxic in relatively small concentrations (Lebrun *et al.*, 1977; Ginevan and Lane, 1978).

The fact that many of the population reductions in litter inhabiting groups occurred in the early part of the growing season was not unexpected since it is well known that SO_2 is highly attracted to moist surfaces (Saunders, 1966; Hocking and Hocking, 1977) and SO_2 toxicity to arthropods can be increased with relative humidity (Lebrun *et al.*, 1978). The soil moisture conditions are wettest in the spring and early summer (Dodd *et al.*, 1978).

Another important possible explanation for the reduction in populations of the more mobile arthropod groups (listed in group two previously) on the fumigated plots may involve behavioral avoidance. Since each treated plot was only 0.52 ha in size, groups such as Staphylinidae, Curculionidae, and Thripidae, which are active fliers, could easily move out of the plots or at least not move into them in their various random movements. Under control conditions, the population of a given species of active arthropod in a given small plot of ground may be maintained by a rough balance of emigration and immigration. This balance could be upset if individuals are repulsed by the presence of SO_2 and hence avoid the plots in their random flight movements. Bromenshenk and Gordon (1978) using the same field sites as this study have shown that the dung beetle *Canthon* sp. (Scarabaeidae) could not be attracted to carrion baits in the SO_2 treated plots in the densities attracted to similar baits on the Control plots (both sites). The differences were significant and suggest that the beetles' behavior was influenced by the SO_2. The critical factor in this explanation is the size of the field plots. The effect of SO_2 on the active arthropod groups may be quite different if a large enough region were exposed so as to rule out behavioral avoidance of the polluted atmosphere. Normal behavioral patterns may or may not be affected to the extent of significantly changing the population size and/or dynamics of a given arthropod species. Hillman and Benton (1972) found reduced foraging activities of honey bees when exposed to SO_2 in low concentrations of 100 to 600 pphm. The resolution of this question remains to be made.

CONCLUSIONS

Significant population reductions in density and/or biomass were observed on SO_2 treated plots during the two-season study although the reductions were not large enough or involve enough of the dominant groups to cause a significant change in the overall total aboveground arthropod population estimated of either site. The arthropod groups which showed significant density and/or biomass changes are listed in Tables 16.3 and 16.4. Inconsistencies between site-years are cause for concern about the extent of the effects of SO_2 fumigation. However, the numerous significant population reductions are accepted as evidence that SO_2 did have a deleterious effect on the above ground arthropod community.

The arthropods listed in Tables 16.3 and 16.4 can be generally categorized into two groups. One group would include the relatively immobile types that are strongly associated with the soil surface litter (Acarina, Diplura, Collembola, and Diptera larvae). The other group would include the relatively mobile, flying insects which may only partially be associated with the surface litter or not at all. The former group members possibly are reduced by direct toxicity of SO_2 or toxic effects on food resources or a change in soil acidity. The second group, in addition to being affected by toxicity (direct or indirect), may also be reduced by behavioral changes due to SO_2 which may result in a change in emigration/immigration ratios, *i.e.*, avoidance of the relatively small field plots. There is published evidence to support both concepts, but our data are not definitive enough to conclude if either or both mechanisms were involved.

REFERENCES

Bromenshenk, J. J. and C. C. Gordon. 1978. Terrestrial Insects Sense Air Pollutants. In: Conference Proceeding, Forest Joint Conference on the Sensing of Environmental Pollutants, 1977. Am. Chem. Soc., Wash., D.C. pp. 66-70.

Dodd, J. L., J. W. Leetham, T. J. McNary, W. K. Lauenroth, and G. L. Thor. 1979. Baseline Characteristics of Producer and Invertebrate Populations and Certain Abiotic Parameters in the Colstrip Vicinity. In: E. M. Preston and T. L. Gullett (eds.) The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report. EPA-600/3-80-044. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 53-106.

Dodd, J. L., and W. K. Lauenroth. 1980. Effects of Low Level SO_2 Fumigation on Decomposition of Western Wheatgrass Litter in a Mixed-grass Prairie. In: E. M. Preston and D. W. O'Guinn (eds.) The Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report. EPA 600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 212-215.

Freitag, R., L. Hastings, W. R. Mercer, and A. Smith. 1973. Ground Beetle Populations Near a Kraft Mill. Can. Entomol., 105:299-310.

Ginevan, M. E. and D. D. Lane. 1978. Effects of Sulfur Dioxide in Air on the Fruit Fly, *Drosophila melanogaster*. Environ. Sci. Technol., 12:821-831.

Heagle, A. S. 1973. Interactions Between Air Pollutants and Plant Parasites. Ann. Rev. Phytopathol., 11:365-388.

Hillman, R. C. and A. W. Benton. 1972. Biological Effects of Air Pollution on Insects, Emphasizing the Reactions of the Honey Bee (*Apis mellifera* L.) to Sulfur Dioxide. J. Elisa Mitchell Sci. Soc., 88:195.

Hocking, D. and M. B. Hocking. 1977. Equilibrium Solubility of Trace Atmospheric Sulfur Dioxide in Water and its Bearing on Air Pollution Injury to Plants. Environ. Pollut., 13:57-64.

Lebrun, P., G. Wauthy, C. Leblanc, and M. Goossens. 1977. Ecologic Test of the Tolerance to SO_2 Toxicity in the Oribatid Mite *Humerobates rostrolamellatus* (Grandjean, 1936) (Acari:Oribatei) Annales Soc. r., Zool., Belg., 106:193.

Lebrun, P., J. M. Jacques, M. Goossens, and G. Wauthy. 1978. The Effect of Interaction Between the Concentration of SO_2 and the Relative Humidity of Air on the Survival of the Bark-living Bioindicator Mite *Humerobates rostrolamellatus*. Water, Air, and Soil Pollu., 10:269-275.

Leetham, J. W. 1975. A Summary of Field Collecting and Laboratory Processing Equipment and Procedures for Sampling Arthropods at Pawnee Site. U.S./IBP Grassland Biome Tech. Rep. 284. Colorado State Univ., Ft. Collins. 49 pp.

Leetham, J. W., J. L. Dodd, and W. K. Lauenroth. Effects of Low-Level Sulfur Dioxide Exposure on Decomposition of Western Wheatgrass (*Agropyron smithii*) Litter Under Laboratory Conditions. (Submitted a).

Leetham, J. W., J. L. Dodd, R. D. Deblinger, and W. K. Lauenroth. Arthropod Population Responses in Three Levels of Chronic Sulfur Dioxide Exposure in a Northern Mixed-grass Ecosystem. I. Microarthropods. (Submitted b).

Saunders, P. J. W. 1966. The Toxicity of Sulfur Dioxide to *Diplocarpon rosae* Wolf Causing Blackspot of Roses. Ann. Appl. Biol., 58:103-114.

SECTION 17

RESPONSE OF *MELANOPLUS SANGUINIPES* TO LOW-LEVEL SULFUR DIOXIDE EXPOSURE FROM EGG HATCH TO ADULT (ORTHOPTERA:ACRIDIDAE)

J. W. Leetham, J. L. Dodd, J. A. Logan, and W. K. Lauenroth

ABSTRACT

The effects of low-level SO_2 exposure on the migratory grasshopper *Melanoplus sanguinipes* (Fab.) from egg hatch to egg-laying adult were investigated by rearing the grasshoppers in controlled laboratory environments. A nondiapausing strain of *M. sanguinipes* was reared from eggs in control and SO_2 environments, the SO_2 concentration being 17 pphm (468 $\mu g \cdot m^{-3}$). No significant difference in egg-hatching success, mean developmental time for each nymphal instar, adult dry weight biomass, and egg production per female per day were found between control and SO_2-exposed individuals. There was, however, a significant reduction in the variability around the mean developmental time for instars three, four, and five in the SO_2-exposed nymphs. At the same time, there was evidence of increased mortality of SO_2-exposed nymphs. It is postulated that the physiologically marginal individuals of a given instar are more vulnerable to the SO_2 stress and hence are eliminated rather than continue to develop slower than the remaining members of the same instar. There were more slower-developing third, fourth, and fifth instar nymphs in the control group.

INTRODUCTION

Studies of the direct toxicity of SO_2 on arthropods are few and generally do not address the question of the effects of realistic atmospheric SO_2 concentrations on arthropod survival. For example, Weedon *et al.* (1939) studied the deleterious effects of very high SO_2 concentrations on various vertebrate and invertebrate animals. They calculated an LD_{50} for the grasshopper *Melanoplus differentialis* (Thomas) at 10700 pphm for 5 days. Sulfur dioxide has been shown to be effective in high concentrations as an insecticide for stored-grain insect pests (Kanaga, 1956).

Only a few studies have been concerned with low-level SO_2 exposure on arthropods. Hillman and Benton (1972) found reduced brood rearing and pollen collection in honey bee (*Apis mellifera* L.) colonies fumigated with SO_2 concentrations of 0 to 500 pphm over 9- and 14-week periods. Ginevan and Lane (1978) found increased developmental times and decreased survivability of fruit flies (*Drosophila melanogaster* Meigen) exposed to 40 and 70 pphm SO_2 during the larval stage. Lebrun *et al.* (1977) found *Humerobates rostrolamellatus* (Grandjean), a bark-living oribatid mite, very sensitive to SO_2. Exposure to 45 pphm for 2 days resulted in 50 percent mortality in experimental populations. All of these aforementioned three studies used SO_2 concentrations much higher than generally encountered even in highly polluted areas which makes the results questionably applicable to real life situations.

This study was undertaken in hopes of clarifying the results of a field study of the effects of low-level, long-term SO_2 exposure on rangeland grasshoppers in southeastern Montana (McNary *et al.*, submitted). In that study season-long exposure of field plots of native northern mixed-grass prairie were exposed to controlled levels of SO_2 of less than 10 pphm (260 $\mu g \cdot m^{-3}$) throughout the growing season (April-October). Significantly reduced grasshopper populations were found, especially for the species *M. sanguinipes* (Fab.). The mechanism by which the populations were reduced was not resolved between direct toxicity and behavioral changes. The field plots were relatively small (0.52 ha), which would have allowed the grasshoppers to leave the area, or at least not enter the area, because of repulsion by the SO_2. This study was performed to test for direct toxicity of SO_2 to a laboratory strain of *M. sanguinipes* reared under continuous SO_2 exposure from egg hatch to egg-laying adult.

The dynamics of nymphal development and nymphal mortality were of prime interest in this study. Low-level, chronic toxicant exposure may affect developmental rates in two ways. Median time to complete an instar may be altered and/or the variation in developmental rates may be affected. Modification of either the time required to complete a stadium or the variation in developmental rates may have profound ramifications upon an organism's total life system. Taylor (1980) discussed in detail the importance of timing critical life history events in insect population dynamics. Stinner *et al.* (1977) discussed the importance of accurately representing variation in life history events. They further presented an example of variation in developmental rates dramatically affecting individual fitness in a *Heliothis zea* (Boddie) population.

Also of interest in this study were adult dry weight biomass (newly emerged) and egg laying success, since both parameters are indicators of the fitness of the individuals within a population. We hypothesized that SO_2 effects on such parameters as food intake rate, digestion rate, and assimilation efficiency would be reflected in the ultimate size and/or egg production of adults. If either, or both, parameters are measurably affected by SO_2 exposure, major population level change would be expected.

MATERIALS AND METHODS

This study was conducted at the headquarters buildings of the field research site of the Natural Resource Ecology Laboratory. Since the site is approximately 35 miles northeast of Fort Collins, Colorado, in the Pawnee National Grasslands, it afforded relatively clean ambient air that could be used without scrubbing. Also, the ambient relative humidity is very low, eliminating the need to artificially lower the humidity before circulation through the exposure chambers. Low humidity is considered critical for successful laboratory rearing of the grasshoppers used in this study.

The exposure chambers used were simple continuous-flow, single-pass cabinets measuring 2 ft by 2 ft by 6 ft (61 cm × 61 cm × 183 cm). They were constructed completely of ¼" (0.64 cm) clear plexiglass with large side-opening doors for easy access. Air movement through the chamber was at an approximately linear velocity of 0.46 to 0.91 $m \cdot min^{-1}$, resulting in a complete air turnover once every 1 to 2 minutes. The chamber inlet and exhaust fans were set up to create a slight negative internal pressure to prevent pollutant leakage. Source SO_2 was fed directly into the inlet air stream. Details of the chamber construction and operation are given by Leetham *et al.* (submitted). Sulfur dioxide concentration was maintained at 17 pphm throughout the study. Measurement of the SO_2 was by two methods. A flame photometric sulfur gas analyzer (Meloy Laboratories, Inc., Model SA-160) was used to get accurate point measurements at the outset and conclusion of the study and occasionally during the interim. For continuous monitoring, a technique of chemical absorption (Pararosaniline method) was used to give average concentrations over time (CFR, 1975).

Rearing and adult maintenance was in clear acetate tubes of two sizes, capped with aluminum screen-covered lids. Large tubes (9 cm diameter x 51 cm long) were used for egg hatching and nymphal development, while smaller tubes (5 cm dia. × 20 cm long) were used to pair adults for egg production. The large tubes were held horizontally on an aluminum rack, while the small tubes were held vertically in moist sand within a styrofoam cup.

Two exposure chambers, control and SO_2, were housed in a temperature-controlled room maintained at 35°C. Relative humidity was uncontrolled but averaged 5-15 percent throughout the study except when passing storm fronts temporarily elevated the humidity.

A nondiapausing strain of *M. sanguinipes* was used for this study. Eggs were obtained from the Range Insect Laboratory, Montana State University, Bozeman, where a disease-free culture is maintained. The strain was originally developed at the Saskatoon Research Station of the Canada Department of Agriculture (Pickford and Randall, 1969). Eggs were maintained in moist vermiculite until hatch. All life stages were fed a diet of head lettuce and a dry-mix medium composed of 50 g alfalfa meal, 50 g wheat midlings, 25 g soybean meal, 5 g brewer's yeast, and 11.5 ml corn oil.

The study was initiated by placing eight groups of eggs numbering 100 to 200 eggs per group in each chamber 7 days prior to hatching to test hatching success and to ensure that the nymphs received the desired exposure from the moment of hatching. All newly hatched nymphs were counted at 24-hr intervals, at which time groups of 20 were placed in the larger acetate tubes for rearing to the adult stage. This ensured that the nymphs were all nearly the same age in any given group of 20, making determination of development stage easier. A total of 16 groups of 20 nymphs were placed in each chamber. At 48-hr intervals, each group of 20 nymphs was evaluated for instar structure and mortality until all survivors reached adulthood. From the surviving adults, male-female pairs were set up in the small acetate tubes for egg production. A reserve of males was maintained to replace those that died before the female died. A total of 48 excess male and 48 excess female adults from each chamber were killed by freezing, dried at 65°C for 24 hr and weighed. Egg pods were retrieved and counted at 48-hr intervals. Moist sand from an ephemeral stream bed was used for the oviposition medium. All eggs laid were counted but not maintained for second-generation studies. Egg-laying was continued for 41 days, with 32 pairs in each chamber at the outset.

A preliminary trial study was performed with just eight groups of 20 nymphs in each chamber. Only nymphal mortality and adult egg-laying success data are included here.

Simple t-tests were used to compare the precent egg hatch in each chamber and, at the end of the study, to compare the mean number of eggs per female per day in each chamber. A paired t-test was used to compare mortality rates while a two-way factorial ANOVA (treatment, sex) was used to compare dry weight biomass data. For the developmental data a probit analysis (Finney, 1971) provided a means for testing the effect of the low-level SO_2 atmosphere on both median and variance in developmental rate (rate of development = 1/time to complete a life stage). A probit transformation was made for the cumulative percent of individuals completing each instar. Because experimental replicate (tubes) were treated identically within treatment and replication was entirely a function of experimental convenience (*i.e.*, the grasshoppers were confined in plastic tubes to facilitate counting), the replicates were pooled and probits were computed for the total treatment population. Sample size varied from 250 to 320. The transformed data were then regressed on the elapsed time (from eclosion) to completion of an instar. Median (M) time to completion of an instar was then computed as

$$m = \frac{1}{b}(5 - I) \qquad (1)$$

where b is the slope from the regression line and I is the y-intercept. The standard deviation in time to completion of an instar was estimated from

$$s = \frac{1}{\beta}. \qquad (2)$$

RESULTS AND DISCUSSION

The percent hatch was similar for the Control and SO_2--71.8 percent (S.D. = 18.0 percent) and 67.4 percent (S.D. = 12.6 percent), respectively. The slightly reduced rate in the SO_2 chamber was not significant ($P = 0.05$). These percentages compare favorably with those (55-80 percent) of Pfadt *et al.* (1979), who used the same strain. They also compare favorably with the 53-77 percent found by Pickford (1960), who used a wild strain of the same species. The fact that the eggs were not maintained under treatment conditions throughout their embryological development confounds the apparent lack of SO_2 effect.

From the probit analysis confidence intervals about median development times indicated no statistically significant difference between control and SO_2 nymphs, and no observable trends existed in median developmental times. An analysis of covariance for homogeneity of regression coefficients, however, indicated that the difference between control and treatment variance in developmental rates was highly significant ($P < 0.05$) for the third, fourth, and fifth instars. Data are summarized in Table 17.1.

The variation in developmental rates is significantly and consistently reduced in the SO_2-treatment group (Table 17.1). A possible biological interpretation of the reduction in variation of developmental rates is that physiologically marginal individuals are adversely affected by the SO_2 atmosphere. This interpretation may be logical if the assumption that physiologically less-fit individuals exhibit a retarded developmental rate under normal circumstances is valid. If so, it follows that these physiologically less-fit individuals would be less likely to withstand the additional stress of SO_2 intoxication. This interpretation is consistent with the observed trend of reduced survival in the SO_2-treatment group (Figure 17.1). Further circumstantial evidence to support the conjecture that reduced variation is the result of increased mortality of slow-developing individuals was gained by performing an analysis for the fifth instar, ignoring the end point (100 percent-completed life stage) for the Control group. When the end point (*i.e.*, those individuals with the most retarded developmental rates) was ignored, no significant difference was found between the Control and treatment groups.

The trend of increased mortality in the SO_2 nymphs appears to support the significant reduction in the variation around the mean developmental time for the latter instars. Mortality data for both trials were tested with a paired t-test. In both trials mortality rates were significantly greater in the SO_2-exposed grasshoppers ($P = 0.001$ in both trials). Total mortality was a bit greater in the first trial (36.9 and 41.2 percent for Control and SO_2, respectively) than in the second (30 percent in both Control and SO_2, respectivlly) than in the second (30 percent in both control and SO_2). These figures compare favorably with the 35-39 percent mortality found by Pfadt *et al.* (1979) using the same strain of grasshopper and nearly the same rearing conditions. The rearing conditions other than SO_2 appear not to have been stressful on the nymphal instars such that greater than normal mortality occurred.

Pfadt *et al.* (1979) concluded that rearing temperature had a substantial effect on the number of nymphal instars that *M. sanguinipes* may have. Six instars usually result when rearing temperatures are below 30°C, and five is common when the temperatures are over 30°C. In their study, 97% of the females and 51% of the males had six instars when reared at 30°C. In this study we found that all grasshoppers had five instars. Our rearing temperature was 35°C.

TABLE 17.1. PROBIT ANALYSIS OF DEVELOPMENTAL DATA

Instar	Treatment	n	I	b*	r^2	m	s	P
3	Control	6	-0.760	0.4441	0.90	12.97	2.25	0.0066
	SO_2	6	-6.279	0.8811	0.99	12.80	1.13	
4	Control	8	-1.746	0.4092	0.95	16.49	2.44	0.0013
	SO_2	10	-6.144	0.6094	0.98	16.84	1.64	
5	Control	6	-5.557	0.4111	0.95	25.67	2.43	0.0270
	SO_2	8	-10.816	0.5924	0.96	26.70	1.69	
5	Control**	5	-8.388	0.5160	0.99	25.95	1.94	0.2642
	SO_2	8	-10.816	0.5924	0.96	26.70	1.69	

n = number of days.

P = probability of obtaining the observed difference in standard deviations by pure chance (0.05 = 5% rejection).

* Note that m is computed from the probit relationship given in equation (1). The apparent inconsistency of increasing the median number of days to completion of the fifth instar control by ignoring the end point is due to an artifact in estimation from the probit equation.

** End point of retarded individuals ignored.

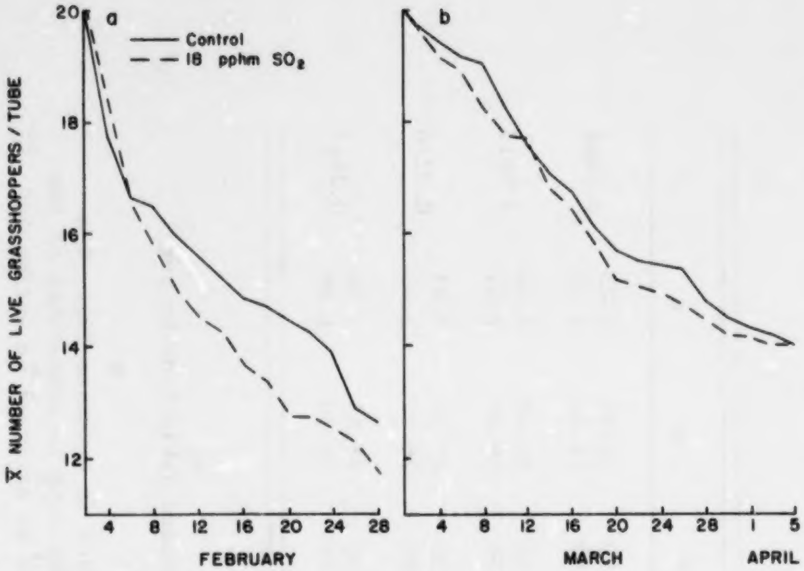

Figure 17.1. Grasshopper survival through five nymphal instars to adult under control and SO_2 exposure. A = preliminary trial, B = second trial.

For adult dry weight no significant treatment effect was found ($P = 0.05$); however, as expected, there was a highly significant ($P = 0.001$) difference between sexes. No significant interaction occurred. Apparently none of the parameters of food intake rate, digestion, and assimilation efficiency were substantially affected.

Egg-laying success for adult females was poor compared with data from Pfadt *et al.* (1979). They found that for adult females held at 30°C and fed a diet similar to that used in this study, an average of 6.7 eggs were produced per female per day. Smith (1966) found an even higher average (9.4) for a wild strain of *M. sanguinipes*. In this study, eggs laid per female per day in the preliminary trial was 0.90 for both control and SO_2-exposed females. In the second trial, there was an increase to 2.3 and 2.0 for the control and SO_2-treated females, respectively. The low egg production resulted from fewer eggs per pod rather than a reduction in the number of pods per female. The fact that egg-laying in both trials was not carried out to completion for all females may have influenced the above calculations. No significant ($P = 0.05$) reduction in egg production was found in either trial. The SO_2 exposure apparently did not confound or add to other, undetermined causes for the overall reduced egg production.

The results of this study help in part to resolve the reason for reduced populations of *M. sanguinipes* in the field study by McNary *et al.* (submitted). However, the implications are such that, unless SO_2 has greater effects on subsequent generations, the increased mortality found here would eliminate only the marginally fit individuals from a population, leaving the healthy individuals to produce an egg supply not substantially different from an unexposed population. In addition, the reduction of some individuals could decrease the intraspecific competition for the remaining individuals. The net result is the maintenance of a field population under regional air pollutant impact much the same as in a similar, unpolluted area. The critical question left unanswered by this study is whether SO_2 can have cumulative effects over numerous successive generations of *M. sanguinipes* or any other insect species.

CONCLUSIONS

Low-level SO_2 exposure of the migratory grasshopper *M. sanguinipes* from egg hatch through egg-laying adult did not cause large effects on most of the parameters of the life cycle measured, including egg-hatch success, rate of nymphal development, adult weight, and adult egg production. Sulfur dioxide did cause a reduction in the variability around the mean developmental time for instars three, four, and five, possibly by increasing mortality in physiologically marginal nymphs. An apparent increase in mortality rate was observed in both trials. Except for egg-laying success, the general rates of the parameters measured in this study agree with those of other life history studies of the same species.

REFERENCES

Code of Federal Regulations, Title 40, Protection of Environment. Part 50.11, July 1, 1975. Office of the Federal Register, General Services Administration, Washington, DC.

Finney, D. J. 1971. Probit Analysis. Cambridge University Press, Cambridge. 333 pp.

Ginevan, M. E., and D. D. Lane. 1978. Effects of Sulfur Dioxide in Air on the Fruit Fly *Drosophila melanogaster*. Environ. Sci. Technol., 12:828-831.

Hillman, R. C., and A. W. Benton. 1972. Biological Effects of Air Pollution on Insects, Emphasizing the Reactions of the Honey Bee (*Apis mellifera* L.) to Sulfur Dioxide. J. Elisha Mitchell Sci. Soc., 88:195.

Kanaga, E. E. 1956. An Evaluation of the Use of Sulfur Dioxide in Fumigant Mixtures for Grain Treatment. J. Econ. Entomol., 49:723-729.

Lebrun, P., T. Wauthy, C. LeBlanc, and M. Goossens. 1977. Ecologic Test of the Tolerance to SO_2 Toxicity in the Oribatid Mite *Humerobates rostrolamellatus* (Grandjean, 1936) (Acari:Oribatei). Annales Soc. R., Zool., Belg., 106:193.

Leetham, J. W., W. Ferguson, J. L. Dodd, W. K. Lauenroth. Design and Construction of a Simple, Continuous-flow Sulfur Dioxide Exposure Chamber. (Submitted.)

McNary, T. J., D. G. Milchunas, J. W. Leetham, W. K. Lauenroth, and J. L. Dodd. The Effect of Controlled Low Levels of SO_2 on Grasshopper Densities on a Northern Mixed-grass Prairie. (Submitted.)

Pfadt, R. E., Y. Afzali, and J. S. Cheng. 1979. Life History and Ecology of the Nondiapause Strain of the Migratory Grasshopper in the Laboratory. Univ. Wyo. Agric. Exp. Sta. Sci. Monogr. 39, Univ. Wyoming, Laramie. 31 pp.

Pickford, R. 1960. Survival, Fecundity and Population Growth of *Melanoplus bilituratus* (Wlk.) (Orthoptera:Acrididae) in Relation to Date of Hatching. Can. Entomol., 92:1-10.

Pickford, R., and R. L. Randell. 1969. A Nondiapause Strain of the Migratory Grasshopper *Melanoplus sanguinipes* (Orthoptera:Acrididae). Can. Entomol., 101:394-396.

Smith, D. S. 1966. Fecundity and Oviposition in the Grasshoppers *Melanoplus sanguinipes* (F.) and *Melanoplus bivittatus* (Say). Can. Entomol., 98:617-621.

Stinner, R. E., J. W. Jones, C. Tuttle, and R. E. Caron. 1977. Population Mortality and Cyclicity as Affected by Intraspecific Competition. Can. Entomol., 109:879-890.

Taylor, F. 1980. Timing in the Life History of Insects. II. Magnitude and Hedging Factors. Ecology. (In press.)

Weedon, F. R., A. Hartzell, and C. Setterstrom. 1939. Effects on Animals of Prolonged Exposure to Sulfur Dioxide. Contrib. Boyce Thompson Inst., 10:281-324.

POTENTIALLY USEFUL BIOINDICATORS AND BIOMONITORS OF COAL-FIRED POWER PLANT EMISSIONS

SECTION 18

OBSERVATIONS ON TWO LICHEN SPECIES IN THE COLSTRIP AREA, 1979

S. Eversman

ABSTRACT

There was a significant increase in sulfur content ($P<0.05$, linear regression ANOVA) in *Usnea hirta* (L.) Wigg. samples in 1979 and 1977 as distance from Colstrip decreased. Other observations on *U. hirta* and *Parmelia chlorochroa* Tayl. -- respiration rates, chlorophyll content, percentage of plasmolyzed algal cells, rate of photosynthesis -- had no significant linear or logarithmic relationship ($P> 0.05$, linear and logarithmic regression) to distance from Colstrip.

INTRODUCTION

Lichens are used throughout the world as bioindicators of air quality (Ferry, *et al.*, 1973; Hawksworth, 1975-1978; Hawksworth and Henderson, 1978, 1979; Henderson, 1979, 1980). Two primary techniques have been used in identifying and delineating polluted areas: mapping lichen communities near urban and industrial areas, and observing specimens transplanted into polluted areas. Symptoms induced by pollutants in the field are compared with symptoms in laboratory-treated specimens.

The Colstrip lichen study used a slightly different approach since (1) the study began before a pollution source was present and (2) epiphytic lichens are sparse in the Colstrip area. Two native lichen species (*Usnea hirta* (L.) Wigg, an epiphyte on ponderosa pine, and *Parmelia chlorochroa*, a soil lichen) were observed over a period of years at varying distances and directions from the Colstrip coal-fired power plants 1 and 2. The plants began operation in September, 1975 and June, 1976, respectively. Observations of anatomical and physiological states were compared with specimens treated with SO_2 in a field fumigation system in Powder River County (Eversman, 1978, 1979) 100 km southeast of Colstrip. This report summarizes observations in 1979, and compares 1979 results with those of previous years.

MATERIALS AND METHODS

Usnea hirta samples were collected from 19 ponderosa pine sites 1-70 km from Colstrip (Figure 18.1, Table 18.1). Monitoring sites within 10 km of Colstrip support lichen communities only on north and east-facing trunk bases, so ponderosa pine branches containing *U. hirta* were transplanted to sites P1-P9 and P17-P19 in September 1975 or April 1976. All observations 1976-1979 were from these transplanted specimens. Source of transplants was Site P10, an east-facing slope 51 km southeast of Colstrip. All sites in Custer National Forest, P10-P16, and site P8 on the Northern Cheyenne Indian Reservation have sufficient native population to use. Sites P8, P15, and P16 received transplants for observation of effects of transplanting. Native *P. chlorochroa* was collected from four grassland sites near EPA exclosures (G1-G4), three other sites near ponderosa pine sites (P3, P4, P18), and the control grassland site (G7).

Samples were washed with distilled water and stored in air-dry conditions in the dark at room temperature for no more than 3 weeks before laboratory observations. Respiration rates were determined manometrically for 250 mg samples at 20°C in saturated condition in the dark.

Chlorophyll extracts were made in two ways. (1) 300-mg samples were extracted with 10 ml boiling methanol, filtered, returned to 10 ml levels, then read at 665 nm on a Beckman DU spectrophotometer. (2) Total pigment and percentages of chlorophyll and phaeophytin were obtained using the chromatography methods and formulas of Brown and Hooker (1977). This was an attempt to decrease chlorophyll degradation to phaeophytin by lichen acids.

Plasmolysis of algal cells was determined by making wet mounts of thallus tips, then recording the number of yellow, plasmolyzed algal cells out of 100 cells on each of three slides (300 cells per sample were counted). Handling of specimens in this way also allowed for close observation of color and integrity of thallus. (SO_2-exposed lichens became crumbly and yellow; untreated specimens were firm and green.)

Sulfur contents were determined by the Montana State University Soil Testing Laboratory, using a dry ash and turbidometric procedure (pers. comm.).

Photosynthesis rates were determined by drying samples to 50 percent of saturated weight, as determined by drying curves, then placing them in flasks in a Gilson respirometer at 10°C in light for 1 hour. Atmosphere samples (10 ml) were removed from the flasks with syringes, and injected into an infrared gas analyzer to determine amount of CO_2 consumed. Controls were respirometer flasks under the same temperature and light conditions, with no lichen samples.

Statistical analysis was through the Montana State University Statistical Center. Programs used were multiple regression; one-way analysis of variance; Newman-Keuls Q, comparison of means; and log transformations (Lund, 1980).

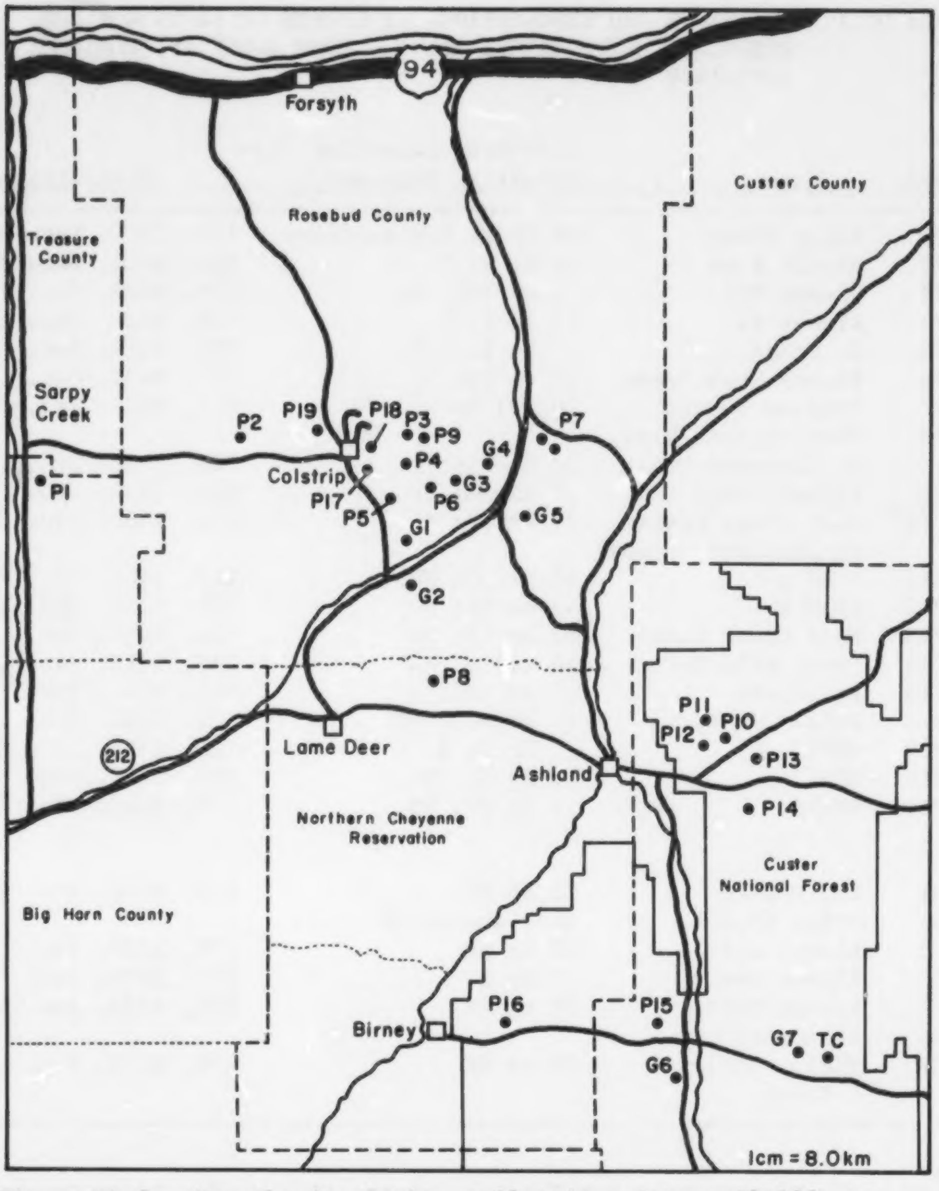
94
Forsyth
Custer County
Rosebud County
Treasure County
Sarpy Creek
P2
P19
P18
P3
P9
P4
P7
G4
G3
Colstrip
P17
P1
P5
P6
G5
G1
G2
P8
P11
P10
P12
P13
P14
212
Lame Deer
Ashland
Northern Cheyenne Reservation
Custer National Forest
Big Horn County
P16
P15
Birney
G7
TC
G6
1cm = 8.0km

Figure 18 .1. Map showing lichen collection sites. P1-P16 are ponderosa pine sites with *Usnea hirta;* G1-G7 are grassland where *Parmelia chlorochroa* was collected. Sites P8, P15, and P16 have both native and transplanted *U. hirta*. Site P7 has two buttes. *U. hirta* was collected from the top and bottom of a hill at P14. P10* = *U. hirta* transplant source. TC = Taylor Creek fumigation sites (ZAPS).

Table 18.1. LOCATIONS AND DESCRIPTIONS OF LICHEN COLLECTION SITES. ROSEBUD, POWDER RIVER, AND BIG HORN COUNTIES, MONTANA; 1975-1979

Number	Name	Distance, Direction from Colstrip, Exposure	Description
P1	Sarpy Creek	48 km W1 ENE exposure	T2N, R37E, Sec. 36 (BH)*
P2	Castle Rock	16 km W; E	T1N, R41E, Sec. 36 (R)
P3	Kluver NE1	7 km ENE; SW	T2N, R42E, Sec. 16 "
P4	Kluver E1	5 km E; W	T2N, R42E, Sec. 29 "
P5	D. McRae	7 km S; NNW	T1N, R42E, Sec. 36 "
P6	Kluver West trees	10 km SE; NW	T1N, R42E, Sec. 2 "
P7	Diamond Buttes	20, 21 km NE; SW	T2N, R43E, Sec. 22 "
P8	Morning Star View, N. Cheyenne Res.	26 km SSE; N	T2S, R41E, Sec. 12
P9	Kluver, near P3	7 km NE; SW	T2N, R42E, Sec. 16 "
P10*	East Otter Creek (transplant source)	51 km SE; ESE	T2S, R46E, Sec. 24 (PR)
P11	SEAM 1	45 km SE; NW	T2S, R46E, Sec. 22 "
P12	SEAM 2	45 km SE; NW	T2S, R46E, Sec. 22 "
P13	Home Creek Butte	55 km SE; NW	T2S, R46E, Sec. 4 "
P14	Three Mile Butte	58 km SE; NW	T4S, R47E, Sec. 10 "
P15	Ft. Howes	70 km SSE; N	T6S, R45E, Sec. 19 "
P16	Poker Jim Butte	66 km SSE; NW	T6S, R44E, Sec. 17 (R)
P17	BNW#1	1 km S; N	T2N, R41E, Sec. 34 "
P18	BNW#2	2 km SE; N	T1N, R41E, Sec. 3 "
P19	BNW#3	1 km NW; SE	T2N, R41E, Sec. 28 "
G1	Hay Coulee	11 km SE	T1N, R42E. Sec. 28 (R)
G2	McRae Knolls	Site abandoned	"
G3	Kluver West	12 km SE	T1N, R42E, Sec. 2 "
G4	Kluver North	5 km E	T1N, R43E, Sec. 6 "
G5	Kluver East	21 km SE	T1N, R43E, Sec. 15 "
G6	Abandoned		
G7	Field, Taylor Creek	98 km SE	T7S, R47E, Sec. 3 (PR)

*BH = Big Horn County; R = Rosebud County; PR = Powder River County

RESULTS AND DISCUSSION

Respiration Rates

Observations from the field SO_2 fumigation sites (ZAPS plots, Eversman, 1978, 1979) indicated that respiration rates of the lichens could be expected to rise when samples were slightly stressed and to fall significantly when subjected to continuous higher SO_2 stress. Therefore, either of these responses was watched for, particularly in samples within a few km of Colstrip.

Variation in respiration rates of *U. hirta* among sites was significant in 1979 (ANOVA, $P<0.05$), but was generally not related to distance from Colstrip with one possible exception (Figure 18.2). *U. hirta* transplanted in 1976 to one of the sites 1 km from the Colstrip power plant (BNW#1) had a significantly elevated respiration rate July, 1979 (ANOVA, $P<0.05$). The same phenomenon was observed at this site in September, 1977, and at site P18 (BNW#2, 2 km from the power plant) September, 1977 and September, 1979. It appears that these two sites may be affected by the power plants.

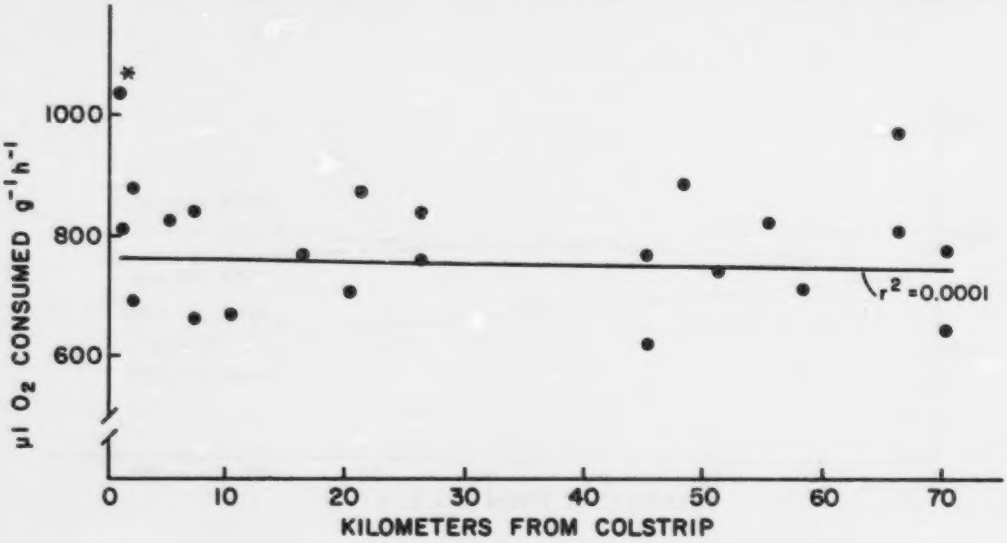

Figure 18.2. Linear regression of respiration rate of *Usnea hirta*, July, 1979, with distance from Colstrip. Each point represents the mean of three to six samples. Regression line was computed using all readings comprising means. Distance from Colstrip was not significant ($P>.05$, linear and log transformation), although point with * (Site BNW#1, 1 km from Colstrip) was significantly higher ($P<.01$, ANOVA) than other sites.

Regression lines comparing 1976-1979 (July respiration rates only) are presented in Figure 18.3. In 1977 and 1978, respiration rates showed a significant linear relationship to distance from Colstrip (regression analysis of variance $P<0.05$, both linear and with log transformation); however, since the slopes of the lines are opposite, the meaning of this relationship is unclear. In 1979, when impact would be expected to have been greater than in 1977 or 1978, the relation between respiration rates and distance was not significant ($P<0.05$, linear regression and log transformation, with analysis of variance). Sample size was greatest in 1979; sample sizes may account for some differences.

All analyses of respiration rates of *P. chlorochroa* gave results that were not significant; sites were not significantly different from each other (ANOVA) and respiration rates were not related to distance from Colstrip (regression). According to ZAPS experiments in 1976 *P. chlorochroa* was almost as sensitive to SO_2 as *U. hirta* when they were placed side by side about 50 cm above the ground (Eversman, 1978, 1979). It could be that SO_2 was not reaching ground level at the grassland sites, either because it is being filtered out by grasses and forbs above ground level, or because the plume from Colstrip was above even grass and forb level at the grassland sites.

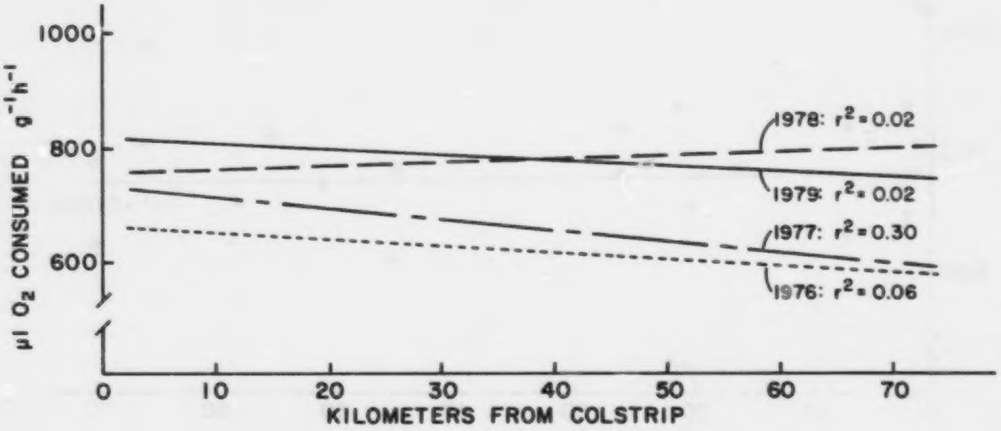

Figure 18.3. Linear regressions of *U. hirta* respiration rates, 1976-1979, month of July only, with distance from Colstrip. Distances were not significant in 1979, but were significant in 1978 and 1977 ($P<0.05$, regression analysis of variance). All recorded respiration readings during July of each year were used for regression computations. Sample size was greatest in 1979 (100 data points) vs. 80 in 1978, 26 in 1977, and 35 in 1976. Variation in sample size may account for some of the differences.

Sulfur Content

The MSU Soil Testing Laboratory determined percentage of sulfur content of lichen samples in 1975, 1977 and 1979. During these years, the laboratory changed their analysis methods so comparisons between years are invalid until intercalibrations are available. Within-year comparisons are possible (Figure 18.4). In 1979, *U. hirta* from the two closest sites to the Colstrip plant had higher sulfate content than samples from other sites, but differences were not significant (ANOVA, Newman-Keuls Q test). However, sulfate content of *U. hirta* was related linearly to distance from Colstrip in 1979 (regression analysis of variance, $P<.05$).

In 1977, with seven sites sampled for sulfur content (P1-P6, P10-P13), there was also a significant linear relationship between distance from Colstrip and sulfur content of *U. hirta* tissue ($r^2 = 0.18$), $P<.05$, linear regression analysis of variance). In 1975, before power plant operations began, no linear relationship existed. *Usnea hirta* samples seemed to be accumulating more sulfur in the Colstrip vicinity than in sites farther away (in Custer National Forest).

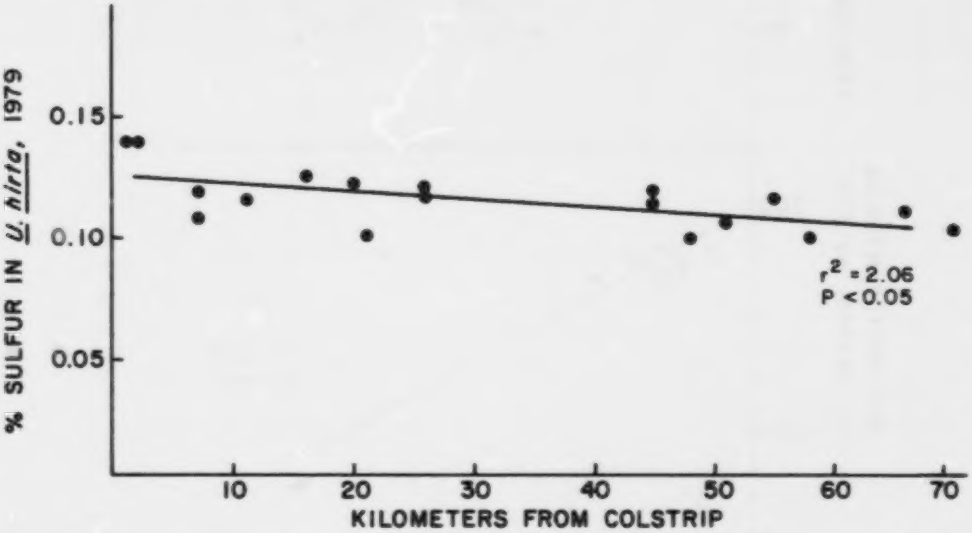

Figure 18.4. Regression of percentage sulfur (as sulfate) of *U. hirta* samples, 1979. Each point is the mean of three to 12 samples. Regression was computed using all readings comprising means. Increased sulfur content was linearly related to distance from Colstrip (negative slope, $P<.05$, regression analysis of variance).

Parmelia chlorochroa samples all had a sulfate content between 0.06 and 0.08 percent in 1979, regardless of location. There were no significant differences among sites and there was no linear relationship between sulfur (as sulfate) content and distance from Colstrip in 1975, 1977, or 1979.

Plasmolysis of Algal Cells

Counting plasmolyzed algal cells gave an immediate impression of total thallus health and integrity, as well as a quantitative measure of presumably viable photosynthesizing algal cells (Figure 18.5). It is perhaps a less objective method than chlorophyll content determination or apparent photosynthetic rate, but it does not consume much sample material nor does it require careful manipulation of temperature, light conditions and water content.

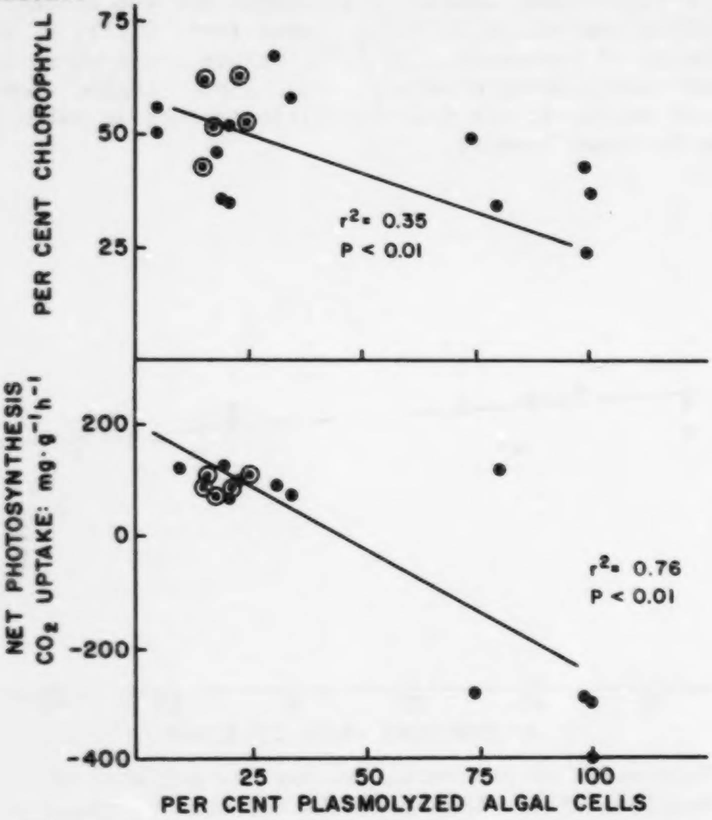

Figure 18.5. Linear regressions of plasmolysis with percentage chlorophyll (acetone wash technique), and plasmolysis with net photosynthesis of *U. hirta*. Both relations were highly significant ($P<0.01$, regression analysis of variance). Data from ZAPS samples (points without circles) were used in computing the regressions.

Regression lines of plasmolyzed algal cells of *U. hirta* versus distance from Colstrip had negative slopes in both 1979 and 1978, but relationships were not significant (Figure 18.6.).

Plasmolysis of algal cells in *P. chlorochroa* was not related to distance from Colstrip, nor were there significant differences in mean plasmolysis among sites in 1979 (ANOVA).

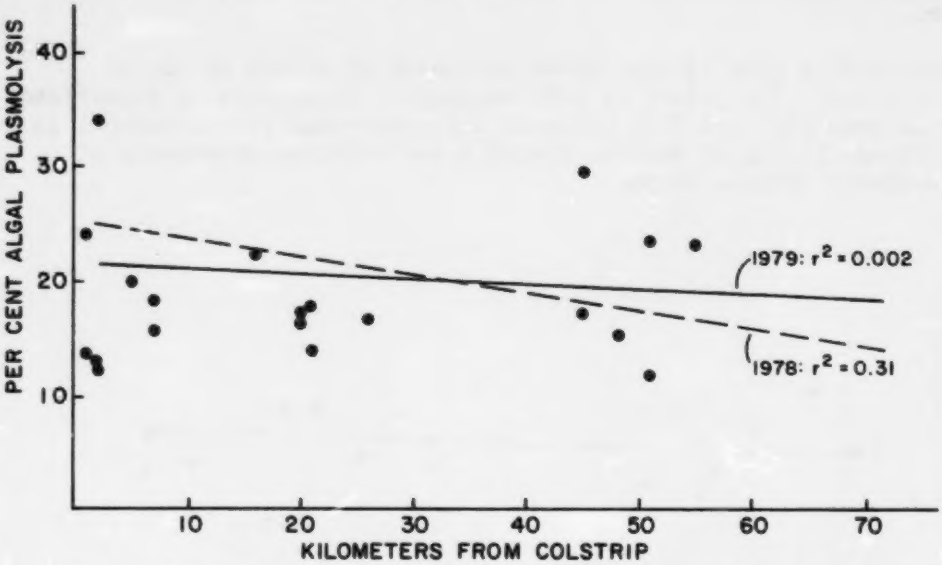

Figure 18.6. Linear regression of percentage of algal plasmolysis of *U. hirta* with distance from Colstrip. Points represent means of three to nine samples collected during 1979. Regression lines were computed using all data points comprising means. Plasmolysis was not significantly related to distance from Colstrip in either 1978 or 1979 (regression analysis of variance).

Chlorophyll Content

Until September, 1979, chlorophyll content of lichen samples was determined by a simple extraction in which lichen materials were boiled in methanol for a few minutes, filtered, brought back up to 10 ml with methanol, then read turbidometrically in a Beckman DU spectrophotometer. Several wavelengths were read, but results were reported as relative absorbance at 665 nm. This method actually included chlorophylls, plus phaeophytin and any methanol-soluble materials.

It was suggested that the presence of lichen acids destroys chlorophyll, removing magnesium and forming phaeophytin, thus giving distorted readings on effects of SO_2 on chlorophyll (Brown and Hooker, 1977; Nash, pers. comm.).

Successive washes in acetone would remove the acids and cause less degradation of chlorophyll due to acid presence. The biggest advantage I found of successive washes in acetone was to allow better separation of pigments (chlorophyll and phaeophytins) during chromatography, leading to ability to quantify amounts of each pigment present. The acetone washes did not completely remove acids, as detected by addition of paraphenylenediamine to the last wash, nor was there any apparent beneficial effect on chlorophyll degradation.

The two methods gave similar gross estimates of effect of SO_2 on chlorophyll content (as judged by ZAPS samples). There were no significant relations between distance from Colstrip and percentage of chlorophyll in extracts (Figure 18.7), or between distance and relative absorbance of methanol extracts (Figure 18.8).

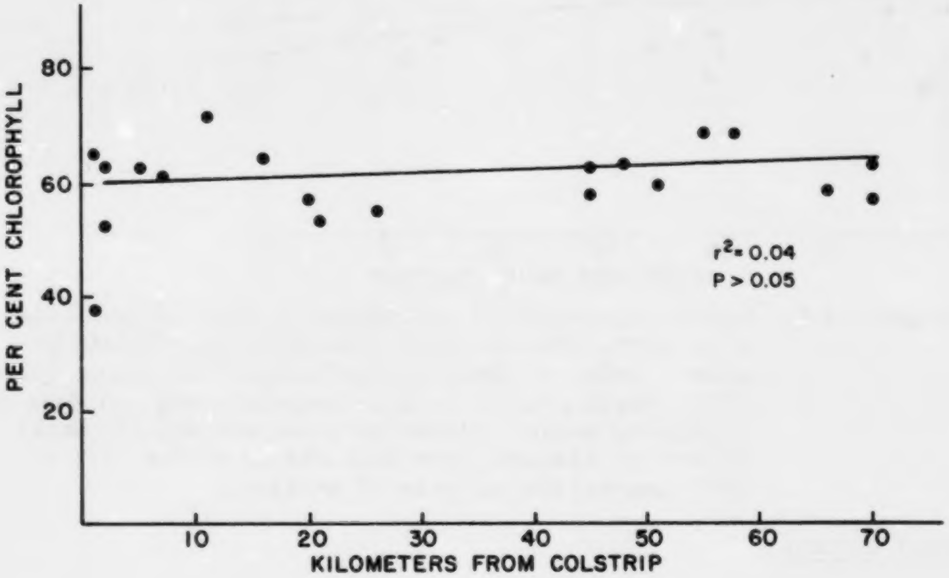

Figure 18.7. Regression of percentage of chlorophyll in pigment extracts of *U. hirta* with distance from Colstrip. Points are means of three samples. Regression line was computed using all observations during July, 1979. This method of chlorophyll extraction (acetone washes, chromatography) gave results similar to methanol extraction method (Figure 18.8).

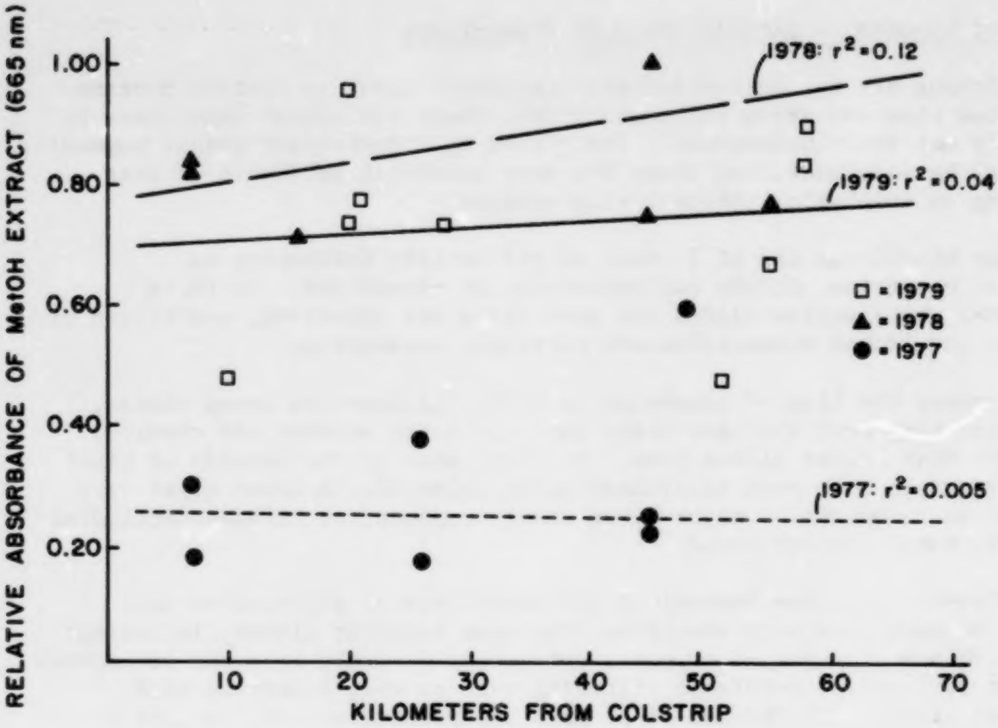

Figure 18.8. Linear regressions of relative absorbance of chlorophyll extracts at 665 nm, 1977-1979, July readings only. Differences in pigment amounts are not linearly related to distance from Colstrip ($P>0.05$, regression analysis of variance).

CONCLUSIONS

The only measured parameter that showed significant relationship ($P<0.05$, regression analysis of variance) to distance from Colstrip was sulfur content of *Usnea hirta* in 1977 and 1979. Its significantly higher level in the immediate Colstrip vicinity as compared with previous years and with Custer National Forest sites indicated some power plant effects but still very slight at this time. Samples collected from all sites appeared "normally" healthy and appropriately green in 1979 as in earlier years. Any possible impact on lichens has been very slight to date, as was expected because of reported low concentrations of SO_2, *ca.* 0.10-0.34 pphm monthly averages, at electronic monitoring stations (Ludwick, *et al.*, 1980).

At the end of the 1979 field season, new fresh material was transplanted onto many sites (P2-P7, P18, P19) in anticipation of possible future lichen biomonitoring in the Colstrip area as power plant construction in the entire study area increases.

Value of Lichens as Bioindicators or Biomonitors

Lichens are not an economically important plant in eastern Montana ponderosa pine and grassland communities; their ecological importance is probably not fully understood. Their role in invertebrate animal communities may be important, and there are many anecdotal incidents of deer browsing on epiphytic lichens during winters.

The ubiquitous use of lichens as air quality indicators in European countries, Canada and Japan must be considered. In these countries where sulfur oxides and acid rains are prevalent, conditions of lichens and lichen communities are carefully documented.

Perhaps the line of reasoning is this. Lichens are green plants, therefore they have the same basic photosynthetic enzymes and chemical pathways that higher plants have. In fact, most of the details of plant photosynthesis have been elucidated using *Chlorella*, a green algal very similar to *Trebouxia*, a major green algal component of lichens (including *U. hirta* and *P. chlorochroa)*.

Lichens have some anatomical and physiological differences that appear to make them more sensitive than most vascular plants, including: 1) Absorbance of water, nutrients, and gases, directly from the atmosphere with no soil and/or substrate filtering such as that occurring with vascular plants. 2) Absence of stomata and protective waxy cuticle on outer surfaces to prevent absorption of materials. If the lichen thallus is moist and if pollutants are present, they will probably be absorbed by the lichen. 3) Absence of deciduous parts; materials accumulate indefinitely. 4) No dormant season of the year; their activity depends on available moisture, including dew and melting snow, which means they can accumulate materials throughout the year, not just during a growing season. 5) The symbiotic system, a balance between the alga and the fungus, may be more delicate than the conventional tissue system of vascular plants. If the processes are being impaired in lichens it should serve as a warning that increasing levels of pollutants will probably cause the same problem in economically important vascular plants when their protective mechanisms have been overcome.

Lichens are an inexpensive bioindicator of air quality, and as such it seems reasonable to include their use in the monitoring of air quality.

REFERENCES

Brown, D. H. and T. N. Hooker. 1977. The Significance of Acidic Lichen Substances in the Estimation of Chlorophyll and Phaeophytin in Lichens. New Phytol., 78:617-624.

Eversman, S. 1978. Effects of Low-Level SO_2 on *Usnae hirta* and *Parmelia chlorochroa*. Bryologist, 81 (3):367-377.

Eversman, S. 1979. Effects of Low-Level SO_2 on Two Native Lichen Species. In: E. M. Preston and T. Gullett, eds. The Bioenvironmental Impact of a Coal-Fired Power Plant, 4th Interim Report. EPA-600/3-79-044. U.S. Environmental Protection Agency. Corvallis, Oregon. pp. 642-672.

Ferry, B. W., M. S. Baddeley, and D. L. Hawksworth. 1973. Air Pollution and Lichens. University of Toronto Press.

Hawksworth, D. L. 1975. Literature on Air Pollution and Lichens II. Lichenologist, 7(1):62-66.

Hawksworth, D. L. 1975. Literature on Air Pollution and Lichens III. Lichenologist, 7(2):173-177.

Hawksworth, D. L. 1976. Literature on Air Pollution and Lichens IV. Lichenologist, 8(1):87-91.

Hawksworth, D. L. 1976. Literature on Air Pollution and Lichens V. Lichenologist, 8(2):179-182.

Hawksworth, D. L. 1977. Literature on Air Pollution and Lichens VI. Lichenologist, 9(1):77-82.

Hawksworth, D. L. 1977. Literature on Air Pollution and Lichens VII. Lichenologist, 9(2):147-152.

Hawksworth, D. L. 1978. Literature on Air Pollution and Lichens VIII. Lichenologist, 10(1):95-100.

Hawksworth, D. L. and A. Henderson. 1978. Literature on Air Pollution and Lichens IX. Lichenologist, 10(2):227-230.

Hawksworth, D. L. and A. Henderson. 1979. Literature on Air Pollution and Lichens X. Lichenologist, 11(1):91-96.

Henderson, A. 1979. Literature on Air Pollution and Lichens XI. Lichenologist, 11(2):313-319.

Henderson, A. 1980. Literature on Air Pollution and Lichens XII. Lichenologist, 12(1);145-148.

Ludwick, J. D., D. B. Weber, K. B. Olsen and S. R. Garcia. 1980. Air Quality Measurements in the Coal-Fired Power Plant Environment of Colstrip, Montana. In: E. M. Preston and D. W. O'Guinn, eds. The Bioenvironmental Impact of a Coal-Fired Power Plant, 5th Interim Report. EPA-600/3-80-052. U.S. Environmental Protection Agency, Corvallis, Oregon. (In Press) pp. 1-19.

Lund, R. E. 1980. A User's Guide to MSUSTAT: An Interactive Statistical Analysis Package. Technical Report. The Statistical Center, Department of Mathematical Sciences, Montana State University, Bozeman. 74 pp.

SECTION 19

EFFECTS OF LOW-LEVEL SO_2 ON TWO NATIVE LICHEN SPECIES: 1979 ZAPS OBSERVATIONS AND PROJECT SUMMARY

S. Eversman

ABSTRACT

Significant reduction in respiration rate occurred in *Usnea hirta* (L.) Wigg. after about 100 days in a fumigation plot at 2-3 pphm. SO_2. At this same SO_2 level, significant reductions in pigment content and increases in plasmolyzed algal cells occurred within 90 days. At about 5 pphm SO_2, there were significant increases in plasmolyzed algal cells within 60 days, decrease in respiration rates within 60 days, and decrease in pigment content within 90 days. Responses of *Parmelia chlorochroa* Tayl. collected from soil surfaces in the same plots were slight and generally insignificant.

INTRODUCTION

The primary objective of this study was to establish SO_2 dose-response curves for two native lichen species, *Usnea hirta* (L.) Wigg. and *Parmelia chlorochroa* Tayl.; *i.e.*, to establish anatomical and physiological responses of the lichens to given dosages of SO_2 in the ZAPS fumigation plots. Laboratory tests of many researchers have established responses to large doses of SO_2 in short periods of time (LeBlanc and Rao, 1975). I have attempted to determine responses of *U. hirta* and *P. chlorochroa* to the various SO_2 doses of the ZAPS plots.

MATERIALS AND METHODS

Usnea hirta samples were transplanted to four posts in ZAPS plots A, B, and C in June, 1979 (the northwestern post in each plot was not used) by moving entire ponderosa pine branches containing lichen growth from the East Otter Creek site in Custer National Forest, 30 km NE of Ashland (site P10), as in previous years. Collections were made 28, 56, and 90 days after transplanting.

Plot D (High) was not observed in 1979; in previous years within 30-60 days lichens in plot D showed nearly 100 percent mortality of algal cells, complete thallus bleaching, significantly reduced respiration rates, and no photosynthesis. Since findings in the ZAPS sites have been used for comparisons with possible responses to SO_2 in the Colstrip power plant vicinity, the dosages in D plot were unrealistically high. Observations in 1979 concentrated in plots A, B and C, ZAPS I and II.

Parmelia chlorochroa samples from a nearby field were placed at the base of one post per plot (the most northeastern one) and collected only in September (90 days of treatment).

The major moss in the ZAPS plots *(Polytrichum piliferum)* and two *Cladonia* species were collected from each of the plots to check cell condition.

Respiration rates were determined manometrically for 250-mg samples at 20° C in saturated condition in the dark. Chlorophyll extracts were made in 1979 according to the method of Brown and Hooker (1977) described in Section 18. Plasmolysis of algal cells was determined by counting cells in wet mounts (Section 18). Sulfur contents were determined by the Montana State University Soil Testing Laboratory, using a dry ash and turbidometric procedure (pers. comm.). Photosynthesis rates were determined in an infrared gas analyzer (Section 18).

Statistical analyses were through the Montana State University Statistical Center programs: multiple regression; one-way analysis of variance, and Newman-Keuls Q, comparison of means (Lund, 1980).

RESULTS AND DISCUSSION

This report summarizes 1979 field observations, and compares and combines results from this year with previous years to establish lichen responses to low SO_2 exposures over 5-month periods in a northern plains grassland.

Respiration Rates

The pattern of respiration rates of *Usnea hirta* established in previous years was repeated in 1979. Figure 19.1 shows results after 90 days of fumigation in 1979, and results in samples after 92 days of fumigation in 1978 and 96 days in 1976. Ninety days of treatment were usually not enough to establish significant differences in *Usnea* respiration rates between A and B plots. Samples from plot C usually had significantly lower respiration rates than samples from plots A and B after 90 days of treatment (ANOVA, $P<.05$).

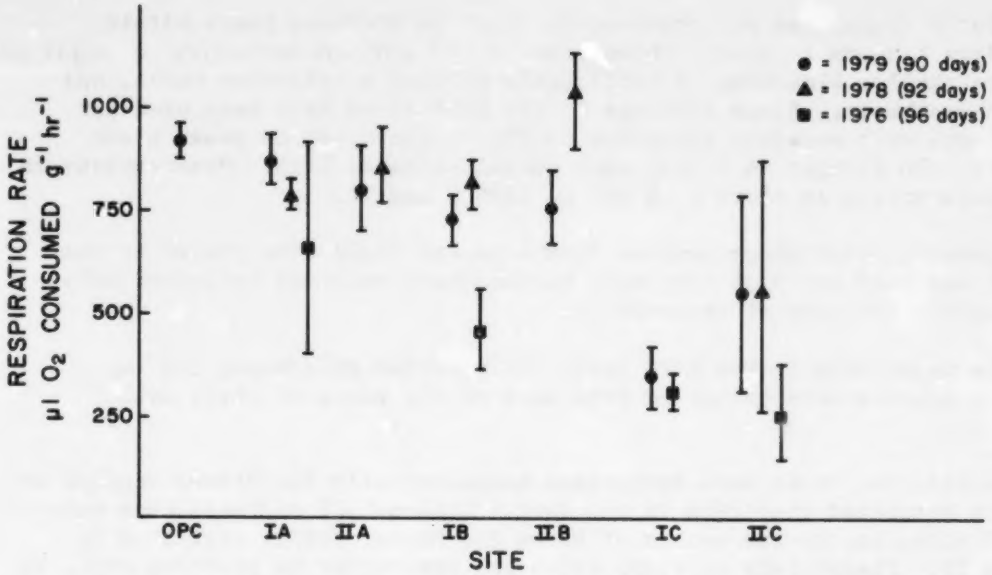

Figure 19.1. Respiration rates of *Usnea hirta*, 1976, 1978, 1979, ZAPS A, B and C (means of 3-9 samples ± .95 confidence interval computed as $t_{0.05}$ x standard error). Results of 90 days exposure in 1979 closely duplicated 92 days exposure in 1978. Respiration rates of *Usnea* from plot C were consistently significantly lower than samples from A and B by 100-110 days of exposure (ANOVA, $P<0.05$). OPC = off-plot control, *ca.* 2 km from ZAPS plots.

The assumption was made that treatment in each plot was essentially the same from year to year. Individual respiration rate readings for *Usnea* were plotted against days for each plot (Figure 19.2). Relation between time in ZAPS I A and II A and respiration rate was not significant (regression ANOVA); the relation was significant in plots B and C, ZAPS I (regression ANOVA, $P<0.05$). There was a significant drop in respiration rate in samples from plot C after 56 days of exposure and after 96 days in plot B (ANOVA), $P<0.05$, Newman-Keuls Q).

Respiration rates of *Parmelia chlorochroa* on the ground showed no significant relationship with ZAPS plots though it responded in a manner similar to *Usnea* when placed 50 cm above the ground (Eversman, 1978, 1979). This position effect is discussed in the following sections. As stated previously (Eversman, 1978) the bacterial populations associated with *Parmelia* probably confound true lichen respiration readings.

Sulfur Content

There were no significant differences in sulfur content among *Usnea* samples from the ZAPS sites after 90 days in 1979 (Table 19.1). While the highest individual readings (0.17 percent, 90 days) and means were from ZAPS B and C, means were not significantly different (ANOVA, $P>0.05$).

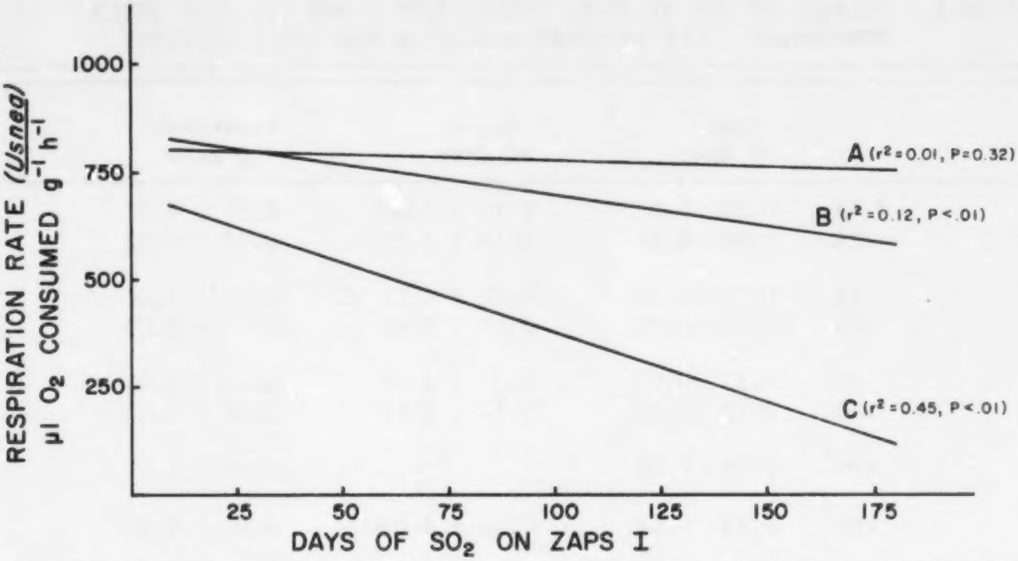

Figure 19.2. Regression of *Usnea* respiration rate with days of SO_2 in ZAPS I. Individual respiration readings at 0, 27, 28, 42, 56, 84, 90, 92, 96, 110, 119 and 156 days in ZAPS I plots 1975-1979 were used in computing regression lines. Samples from plot A were never significantly different from day 0. Samples from B were significantly lower than day 0 after 96 days. Samples from C were significantly lower than day 0 readings after 56 days (ANOVA, $P<.05$; Newman-Keuls Q).

Results in the Colstrip area indicated some significantly higher sulfur content in *Usnea* specimens within 2 km of Colstrip (Section 18) Perhaps the 90 days of exposure in the ZAPS plots were not adequate to accumulate different sulfur amounts. Or perhaps as the *Usnea* samples became less viable they were unable to metabolically accumulate sulfur as healthier specimens do. Gilbert (1969) demonstrated less sulfur accumulation by killed *Usnea* samples than by living ones.

Sulfur contents were not determined for *Parmelia* samples from the ZAPS sites. In previous years differences across plots were not significant.

Plasmolysis of algal cells

The only analytical method that consistently showed significant differences in *Usnea* and *Parmelia* samples between ZAPS plots A and B 1976-1979 was counting plasmolyzed algal cells. Differences became apparent at 30 days and were always significant by 60-90 days (Figure 19.3; ANOVA, $P< 0.05$). Results from *Parmelia* samples taken from the soil surface showed less clear responses; significantly higher plasmolysis rates generally occurred only in plot D after 60 days of exposure (1975, 1976).

TABLE 19.1. SULFUR CONTENT OF *USNEA HIRTA*, ZAPS I AND II, 1979 (MEAN PERCENTAGE ± ONE STANDARD DEVIATION FOR THREE SAMPLES)

	July 28 days	August 56 days	September 90 days
IA	0.10 ± 0.03	0.11 ± 0.01	0.11 ± 0.01
IIA	0.09 ± 0.01	0.11 ± 0.02	0.08 ± 0.02
IB	0.10 ± 0.02	0.11 ± 0.01	0.13 ± 0.04
IIB	0.12 ± 0.02	0.11 ± 0.02	0.11 ± 0.06
IC	0.11 ± 0.02	0.11 ± 0.02	0.12 ± 0.05
IIC	0.11 ± 0.02	0.10 ± 0.01	0.12 ± 0.05
OPC*	0.09 ± 0.02		0.09 ± 0.01
EOC†	0.12 ± 0.02	0.10 ± 0.03	0.11 ± 0.01

*OPC = Off-plot control †EOC = East Otter Creek, transplant source. ANOVA, P = 0.72.

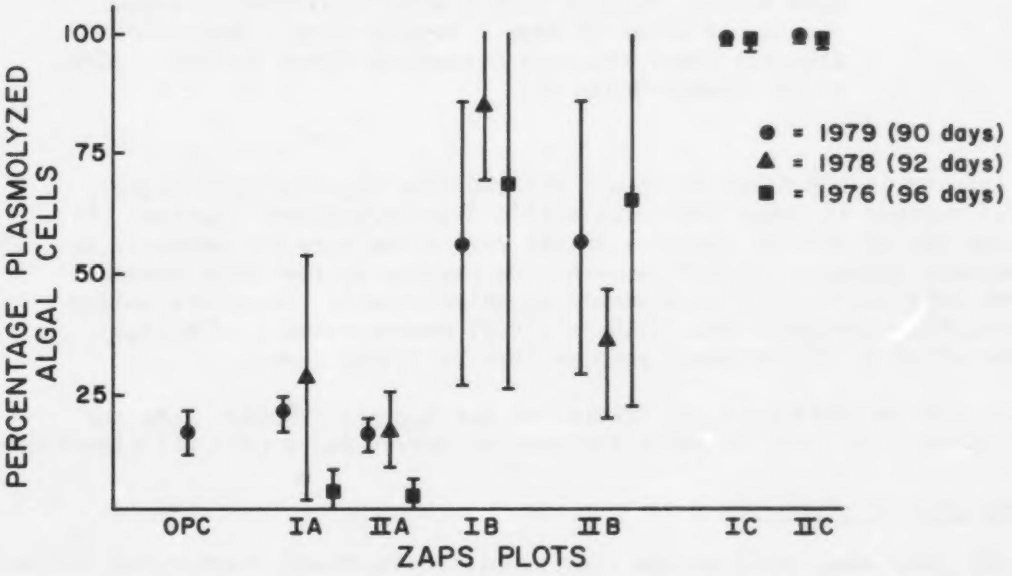

Figure 19.3. Percentage of plasmolyzed algal cells in *Usnea hirta* at 90 days (1979), 92 days (1978) and 96 days (1976 SO_2 exposure, ZAPS I and II. Differences between A, B and C are highly significant (ANOVA $P<0.01$). Each bar is mean ± .95 confidence interval (computed as $t_{0.05}$ x standard error). OPC = off-plot control.

Figures 19.4 and 19.5 illustrate relationships between time in ZAPS I plots and plasmolysis rates. *Usnea* showed a more significant response than *Parmelia;* however *Usnea* was about 50 cm above the ground and *Parmelia* was on the soil surface. Sulfation plate studies (Eversman, 1978; Preston and Gullett, 1979) showed significantly less SO_2 reaching 5-10 cm above the ground (with presumably even less at ground level) than was detected 50-100 cm higher. Reduced *Parmelia* responses, compared with *Usnea*, were assumed to be partly a result of lesser SO_2 levels at the soil surface. *Parmelia* appeared to be slightly less sensitive than *Usnea* (Eversman, 1978), and since it was directly on the soil, substrate buffering may have occurred. *Parmelia* control samples tended to exhibit slightly increased plasmolysis throughout the summer, from spring to autumn, that perhaps contributed to the slope line in Figure 19.5.

Taylor, Leininger, and Hoard (pers. comm.) observed significant reduction of lichen cover in the ZAPS plots C and D since 1976. I collected *Polytrichum* piliferum (moss) and two sterile *Cladonia* (lichen) species from all ZAPS plots in 1979 for cell observations. *Cladonia* plasmolysis means ranged between 10-14 percent in ZAPS A and B, 15-18 percent in C, and over 30 percent in ZAPS D. It seems likely that some SO_2 effects were felt by lichens on the soil surface particularly in plots C and D.

Moss cells appeared "normal" in all specimens and there were no visible differences in moss plants among plots.

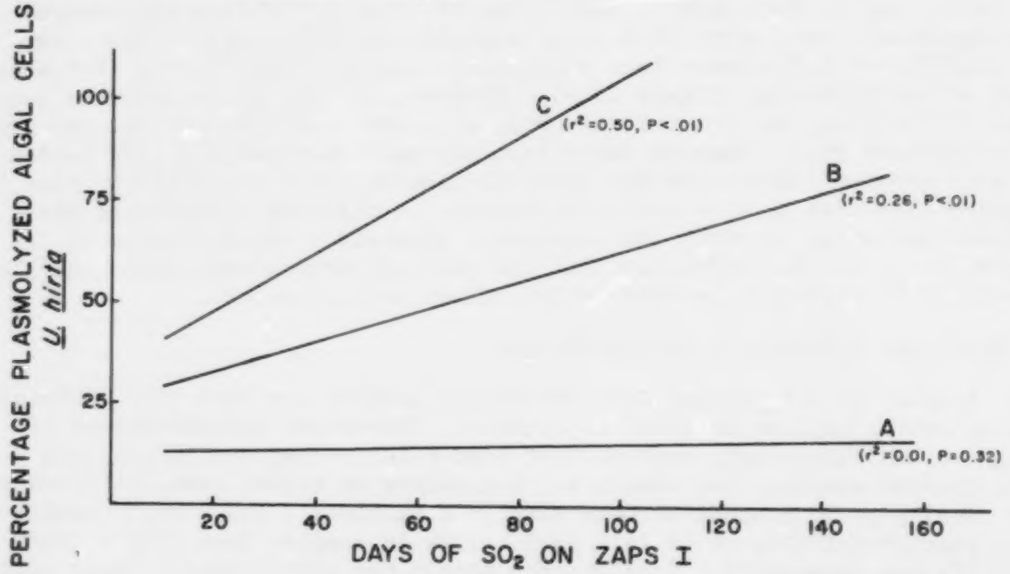

Figure 19.4. Regressions of percentage plasmolyzed algal cells of *U. hirta* with time on ZAPS I, plots A, B and C. Individual plasmolysis readings from 0 (day of transplanting), 27, 28 33, 47, 56, 57, 90, 92, 110 and 119 days in ZAPS I plots in 1975-1979 were used in computing regression lines. Probabilities are from regression analysis of variance.

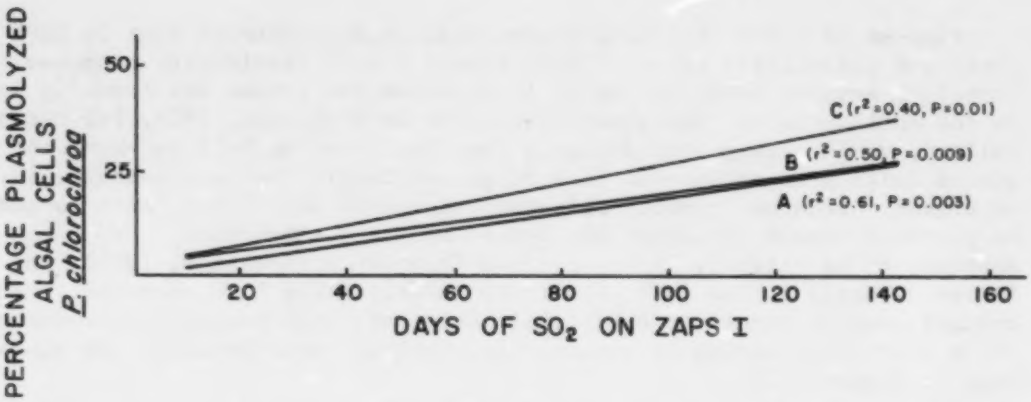

Figure 19.5. Regressions of percentage plasmolyzed algal cells of *P. chlorochroa* with time in ZAPS I, plots A, B and C. Samples were taken from the soil surface. Individual plasmolysis readings from 0 (day of transplanting), 33, 47, 60 and 90 days were used in computing regressions. Probabilities are from regression analysis of variance.

Photosynthesis

Usnea samples from plot C, ZAPS I and II, had a significantly reduced photosynthesis rate after 90 days of exposure in 1979. Again, there were no significant differences between Control samples, samples from plot A and most of the B samples (Figure 19.6). However, one set of three *Usnea* samples from one position in plot B illustrated a common occurrence in samples from this exposure plot. Samples taken from the most southwestern post tended to show greater response to SO_2 than did samples taken elsewhere in plot B. Samples from plot B, when averaged together, consistently exhibited the greater variances in every characteristic measured (respiration rate, plasmolysis, *etc.*), indicating that the various amounts throughout the plot seemed to be threshold between slight effect and pronounced.

Pigment and chlorophyll determinations

Removal of all pigment from the lichen samples has been very difficult using either boiling methanol or acetone. Therefore, determinations of pigment and chlorophyll content have been relative between Control and SO_2-treated samples, not absolute. Regardless of method used, differences in total pigment content between samples from plots A and B were usually not significant (Figure 19.7a); variability in samples from plot B (ZAPS I and II) was pronounced. Samples from plot C had significantly lower total pigment amounts ($P<0.05$, ANOVA) than samples from A and Control plots.

Stressed chlorophyll degrades to phaeophytin, therefore higher levels of chlorophyll were expected in healthy specimens and higher levels of phaeophytin were expected in stressed specimens. However, samples treated with acetone and chromatographed (Brown and Hooker, 1977) showed no significant differences across treatments (Figure 19.7b). Simple pigment extract

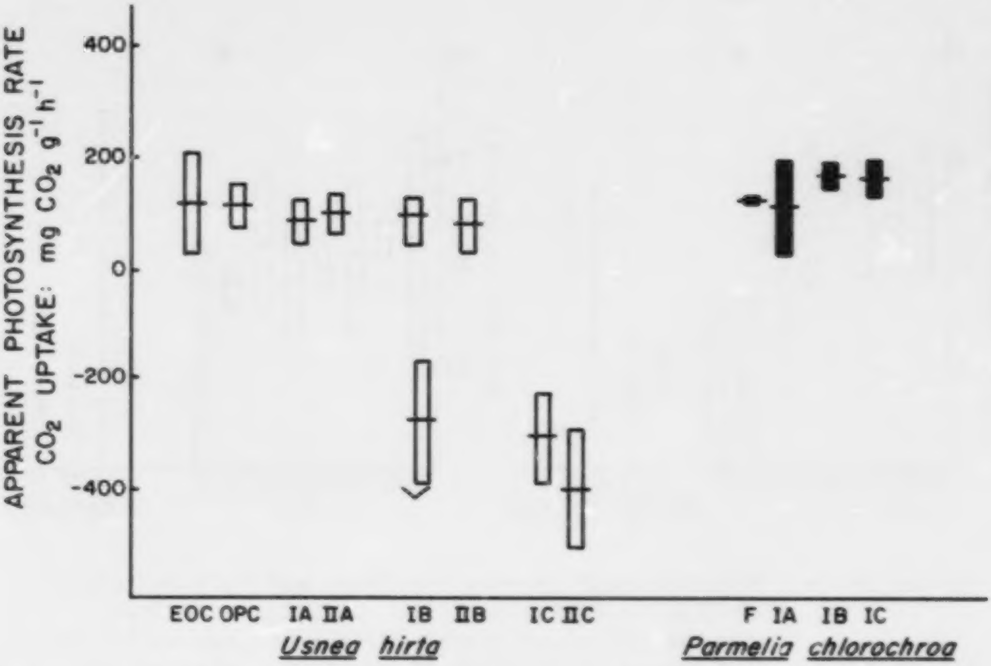

Figure 19.6. Apparent photosynthesis rate of *Usnea hirta* and *Parmelia clorochroa* after 90 days in ZAPS plots, 1979. Each bar is the mean ± .95 confidence interval (computed as $t_{.05}$ x standard error) of three samples. Rates were determined with an infrared gas analyzer at 50 percent saturation. *Usnea* samples from one position in ZAPS IB and plots C had net respiration.

procedures appeared to be somewhat more informative in this case than the more complex chlorophyll/phaeophytin determination methods.

Parmelia samples from the ground in plot C had a significantly higher total pigment content, and a slightly higher chlorophyll content than other samples (Figure 19.7). These results were similar to those obtained in 1977. It appeared that presence of low amounts of SO_2 was stimulating pigment production.

When regressions were computed between percentage of plasmolyzed algal cells and apparent photosynthesis rate for *Usnea* (Figure 19.8), the relationship was highly significant (regression ANOVA, $P < 0.01$). The relation between plasmolyzed algal cells and percentage of chlorophyll (Figure 19.9) was slightly less significant ($P < 0.05$). Counting plasmolyzed algal cells gave not only an observation of gross anatomical appearance, but also an estimate of amount of chlorophyll and photosynthesis rates for *Usnea*.

Results for *Parmelia* were less definitive, with no apparent relation between plasmolysis, chlorophyll content, and photosynthesis.

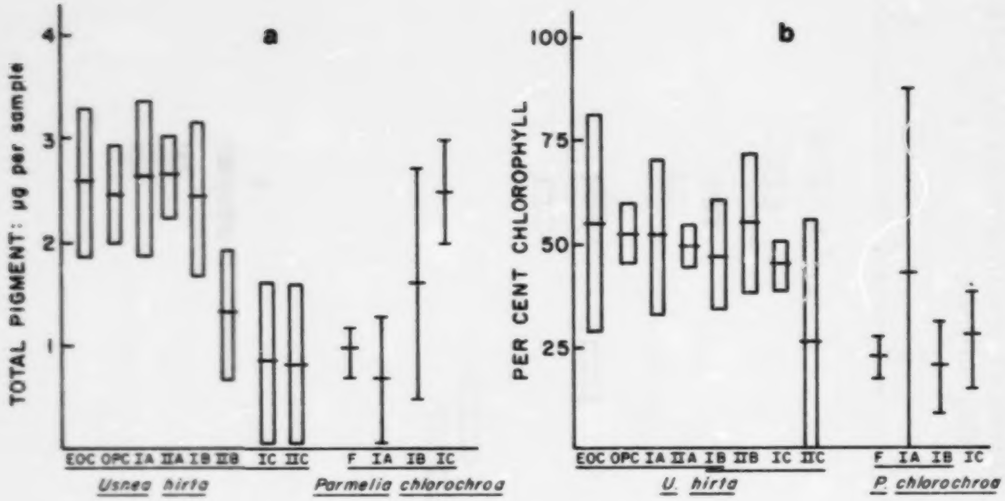

Figure 19.7a. Total pigment content for *Usnea* and *Parmelia* after 90 days in ZAPS plot 1979. Underlines indicate samples not significantly different from each other.

Figure 19.7b. Percentage of chlorophyll in pigment extracts of *Usnea* and *Parmelia* after 90 days in ZAPS plots, 1979. Underlines indicate no significant differences across treatment plots. Bars are means ± .95 confidence interval ($t_{0.05}$ x standard error) for three samples. EOC = East Otter Creek (transplant source). OPC = off-plot control 2 km from ZAPS. F = field, source of *Parmelia* transplants.

CONCLUSIONS

Usnea hirta consistently exhibited better-defined responses to fumigation than did *Parmelia chlorochroa*. There are two possible reasons: 1) The growth form of *Usnea* is bushy (fruticose) and its usual position (tufts on bark of trees) give more surface area for exposure to and absorption of SO_2. *Parmelia* is leaf-shaped (foliose) with proportionately less surface exposed to air. 2) *Parmelia* inhabits soil; *Usnea* is an epiphyte on ponderosa pine. The elevated position of *Usnea* may expose it to more SO_2 and the acidic bark (pH less than 5.0) offers little buffering potential. *Parmelia* was exposed to less SO_2 probably because of vegetational scrubbing of taller plants around it. Limestone-derived soils in many places offer buffering capacity that would decrease SO_2 effects. When *Parmelia* was elevated on ponderosa pine branches with *Usnea* in 1977 ZAPS observations, it was nearly as sensitive as *Usnea* (Eversman, 1978, 1979).

LeBlanc and Rao (1975) suggested that long-range average concentrations of SO_2 above 3 pphm would probably cause acute injury to epiphytes in the Sudbury, Ontario area. Results from the ZAPS sites, C (*ca.* 5 pphm) and D (*ca.* 8 pphm, geometric means) supported this statement. Within 60-90 days

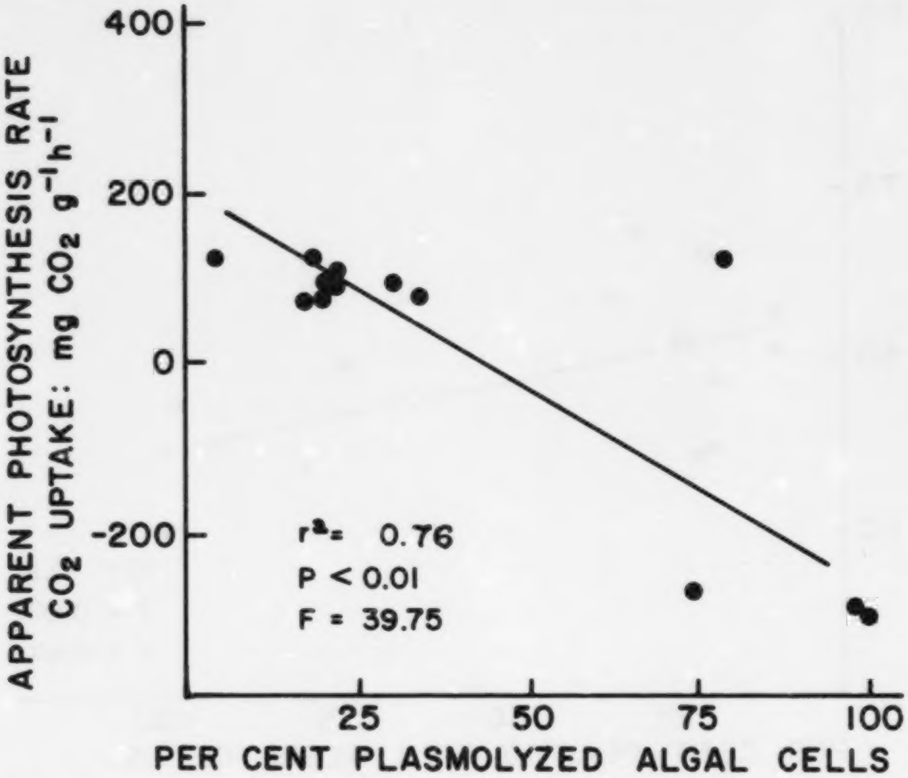

Figure 19.8. Regression between percentage plamolyzed algal cells of *Usnea hirta* and apparent photosynthesis rate. Each dot represents the mean of three samples from ZAPS plot A, B and C. I and II after 90 days, 1979. P and F values are from regression analysis of variance.

thalli were bleached and there were significant decreases in vital processes (photosynthesis, respiration), in pigment content and cell viability, especially in *Usnea hirta*.

LeBlanc and Rao also suggested that long-term SO_2 exposure of 0.6 to 3.0 pphm could cause chronic injury to lichens. After 60 days in plot B (*ca.* 2-3 pphm), *Usnea* had elevated plasmolysis rates, reduced photosynthesis and pigment contents, erratic respiration rates, and visibly bleached thalli. After 90-100 days in plot B, all of these characteristics showed significant differences when compared with samples from Control sites and ZAPS plot A. Depending upon definitions of "acute" and "chronic", samples of *Usnea* and elevated *Parmelia*, always exhibited adverse effects after two to three months in B plots.

ZAPS plot A has been recorded having about 1 pphm SO_2 average (geometric mean)(Preston, pers. comm.). The longest lichen testing period was 156 days

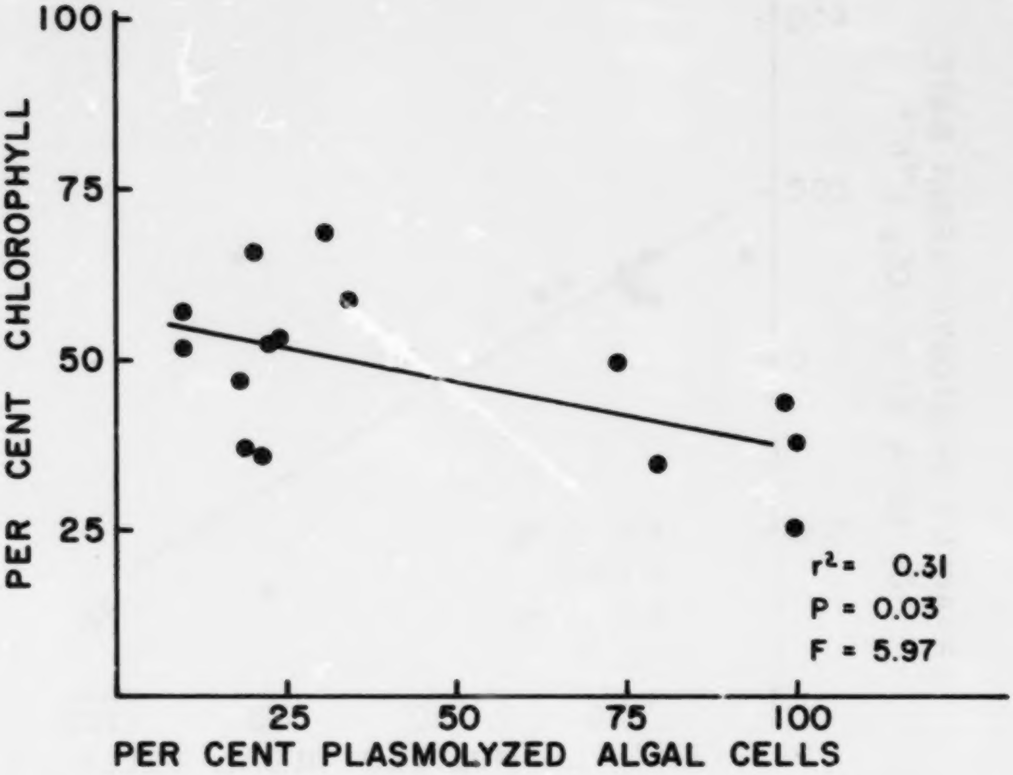

Figure 19.9. Regression between percentage plasmolyzed algal cells and percentage of chlorophyll in pigment extracts for *Usnea hirta*. Each dot represents the mean of three samples from ZAPS I and II, plots A, B and C after 90 days, 1979. P and F values are from regression analysis of variance.

in 1976; the shortest was 84 days in 1978. Within these time periods, there were no significant differences between sampling time and the day of transplanting. The conclusion from plot A is that it would take more than 156 days of constant SO_2 exposure at that recorded level to cause detectable adverse effects in *Usnea hirta* in this climatic regime.

When *Parmelia* was extensively sampled from the soil most detectable responses were from plot D with mostly statistically insignificant responses from plots B and C. The responses that did occur would have been at SO_2 levels less than the monitored amounts since monitoring devices were placed above ground level where SO_2 amounts appear to be higher than directly on the soil surface.

REFERENCES

Brown, D. H. and T. N. Hooker. 1977. The Significance of Acidic Lichen Substances in the Estimation of Chlorophyll and Phaeophytin in Lichens. New Phytol, 78:617-624.

Eversman, S. 1978. Effects of Low-Level of SO_2 on *Usnea hirta* and *Parmelia chlorochroa*. Bryologist, 81 (3):367-377.

Eversman, S. 1979. Effects of Low-Level SO_2 on Two Native Lichen Species. In: Preston, E. and T. Gullett, eds. The Bioenvironmental Impact of a Coal-fired Power Plant. 4th Interim Report. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon pp. 642-672,

Gilbert, O. L. 1969. The Effect of SO_2 on Lichens and Bryophytes around Newcastle upon Tyne. Air Pollut., Proc. Eur. Congr. Influence Air Pollution, Plants and Animals, 1st. 1968. pp 237-272.

LeBlanc, F. and D. N. Rao. 1975. Effects of Pollutants on Lichens and Bryophytes. In: Mudd, J. B. and T. T. Koslowski, eds. Responses of Plants to Air Pollution. Academic Press, New York. pp. 237-272.

Lund, R. E. 1980. A User's Guide to MSUSTAT: An Interactive Statistical Analysis Package. Technical Report. The Statistical Center, Department of Mathematical Sciences, Montana State University, Bozeman. 74 pp.

Preston, E. M. and T. L. Gullett. 1979. Spatial Variation of Sulfur Dioxide Concentrations on ZAPS During the 1977 Field Season. In: Preston, E. and T. Gullett, eds. The Bioenvironmental Impact of a Coal-fired Power Plant. 4th Interim Report. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 306-330.

SECTION 20

FLUORIDE AND ARSENIC CONCENTRATIONS IN HONEY BEES NEAR COLSTRIP

J. J. Bromenshenk

ABSTRACT

Since the coal-fired power plants were put in operation at Colstrip in 1975 and 1976, fluorides in bee tissues have demonstrated significant increases over baselines at apiaries downwind and within 20 km of the plants. In the fall of 1979, fluoride levels at sites having concentrations significantly greater than baselines were similar to 1976, when some levels were more than twice those observed before the power plants began operation. Nearly all fluoride levels for early summer of 1979 were substantially higher than any observed in previous years. In 1977, high concentrations of fluoride were observed in bees at two sites, one north and one south of Colstrip. In 1979, mean fluoride levels at the south site exceeded that of 1977 by a factor of 1.5, an approximate 11 fold increase over baselines. No bees were at the north site in June of 1979. At sites northeast of Colstrip, June/July fluoride concentrations were 3 to 17 times baselines. Levels at one of these sites exceeded reported bee toxicity thresholds. Although fluoride levels varied significantly in 1979, at none of the sites did arsenic levels exceed baselines.

INTRODUCTION

Literature reviews and detailed rationale for selecting honey bees as biological monitors appeared in the preceeding five interim reports of the Colstrip project. Bees serve as bioaccumulators and magnify the levels of many chemicals in their surroundings making it easier to detect the presence and distribution of pollutants. They also provide information about the potential for transfer of pollutants through food chains, especially to humans via honey or pollen. They are manageable social insects that can inhabit almost any biome, and they provide an abundance of sampling material (bees, pollen, honey, and wax). Because bees are beneficial insects in terms of, products (honey, wax, and pollen) and services (pollination), information gathered using a bee monitoring system is directly applicable to human welfare.

Monitoring honey bees should serve as an early warning system of pollutant accumulation and effects which may harm other organisms or alter ecosystem structure and/or function. Hazards to the bees themselves and to the beekeeping industry may also be discovered.

I emphasized fluoride in this study because it is a coal combustion emission, is relatively simple to detect analytically, has low background levels in flora and fauna of native grasslands, and is toxic to bees. Although coal-fired power plants emit substantially more sulfur than fluoride, my previous attempts to examine anthropogenic sulfur in honey bees proved unsuccessful. High background levels of sulfur in bee tissues (presumably in sulfur bonds of proteins) tended to mask any detectable incremental increase (Hillman, 1972).

Honey bees take up large amounts of arsenic near copper smelters and have been reported to take up arsenic emitted by coal-fired power plants (Lillie, 1972). Colstrip bees have been monitored for any long-term build-up of this element, but 1975-78 arsenic levels in these bees, with a few exceptions, have been low.

Air and water are the media whereby pollutants released by activities such as the mining and burning of coal at Colstrip may readily reach bees. Therefore, fluoride concentrations in apiary water supplies and levels of fluoride and sulfur in the ambient air have been monitored concurrently with levels in bees and pollen.

MATERIALS AND METHODS

In 1979, honey bees, pollen, water, and air were sampled at 16 apiaries during late June/early July and again in mid-September. Many of the colonies are transported to California each winter for pollination of orchards and vineyards and are returned to Montana in April and May. Any food supplies (pollen and honey) brought from California were likely consumed and replenished by June. Therefore, contaminants carried back with the "migrant" colonies should be dispersed by the June/July sampling unless fluoride is retained in the beeswax. Also, several population turnovers should have occurred (brood cycles are approximately three weeks). Colonies distant from Colstrip (>40 km) are not moved out of the region and can be used as additional controls. Bees sampled near Colstrip in June/July had collected several boxes (supers) of surplus "sweet clover" honey. This provided further evidence that extensive foraging and nectar gathering had occurred since their return from California.

The autumn (September) collection was performed before the apiaries were transported back to California. The beekeeper moved the colonies from NE 3 and NE 4 to a stockpile location 1 day before they were scheduled to be sampled. I found and sampled these before they were shipped, but it was not clear in all cases which of the marked hives were from which of the two bee-yards. Therefore, the results for these sites for September, 1979, are indicated as NE 3/4.

Bees and Pollen

Each apiary contained seven to 50 colonies; most had 20 to 30 colonies. A high velocity, battery-powered, acrylic vacuum apparatus was used to obtain 30 gms wet weight of bees (about 300 bees) from the entrance of each of 10 hives (Bromenshenk, 1978). At each location, the samples were immediately frozen and stored in Whirl Pacs® at -20°C.

A plastic pick was used to collect pollen from brood chamber combs of 10 colonies at each location. The pollen samples were stored in plastic vials at room temperature. A 100 gm wet weight pooled sample (about 1,000 bees) was obtained at the entrance of every hive in each beeyard to provide a quick screening method of analyses, to produce an average sample from each location, and to ensure sufficient quantities of materials for quality assurance tests, pesticide tests, and other tests.

Sulfation and Calcium Formate Plates

Two sulfation and two calcium formate plates were mounted on posts at the grassland canopy level (75 cm above the ground) in each apiary in June and collected in September in order to measure ambient air concentrations of reactive sulfurs and fluorides. All of the analyses were complete, but statistical examination of the data for 1978 and 1979 was incomplete at the time of this report.

Water

Water was sampled at each apiary, in addition to bees, pollen, and air. All beeyards were located within a few hundred meters of easily accessible water in streams or reservoirs. A minimum of 500 ml of water was obtained at points where bees were landing to drink. Samples were collected and stored in Naglene® bottles and frozen until analyzed. Besides creeks and ponds, any water in livestock watering tanks within 0.5 km of beeyards was also sampled.

Fluoride Analyses

Before being analyzed for fluoride, whole bees and pollen were oven-dried at 45°C for seven days and ground in a Wiley-Mill® to pass a 40-mesh screen. For each sample, 0.5 gm of ground and dried material was placed in a metal crucible and slurried with distilled water with 0.05 gm of reagent grade calcium oxide. The samples were charred under infrared lamps before being ashed in a muffle furnace at 600°C for at least 6 hours. The ashed samples were digested in 2 ml of perchloric acid and subsequently diluted to 100 ml total volume with Orion Tisab® total ionic strength activity buffer. Fluoride determinations were made using an Orion® specific ion probe inserted into the 150 ml beakers containing the dissolved samples which were stirred constantly during analysis. Water was analyzed using the Orion probe and 150 ml of equal parts of water and buffer solution.

Arsenic

Arsenic determinations were carried out by F. F. Munshower and A. R. Neuman, Animal and Range Sciences, Montana State University. One-g samples were weighed into 125 ml Erlynmeyer flasks and digested in 30 ml of a 3:2 moisture of nitric perchloric acid. Samples were left overnight and then heated slowly to solubilize. The heat was gradually increased to reduce sample volume to one-half. The samples were cooled and 10 ml of a 1:1 mixture of nitric:sulfuric acid added. Heat was increased until perchlorate fumes evolved out of the flasks. Heating continued until dense white sulfate fumes occurred. Volume was reduced by heating to 5 ml and the samples allowed to cool. The cooled samples were transferred to 50 ml volumetric flasks containing 15 ml of concentrated hydrochloric acid, 10 ml of water, and 1 ml of 1 percent (w/v) potassium iodide. Cooling to ambient temperature and allowing the samples to stand for 1 hour allowed reduction of As^{+5} to As^{+3}.

Standards were prepared in 100 ml volumetric flasks which contained 30 ml hydrochloric acid, 2 ml of potassium iodide, and 5, 15, and 20 mg of arsenic. These were allowed to stand for 1 hour prior to analyses. The coefficients of determination for the results of analyses for arsenic in the standards approximates 0.997, indicating good recovery of the chemical.

All determinations were made using an atomic absorption spectrophotometer connected to an arsine generator. The analytical procedure consisted of placing a 20.0 ml aliquot of standard/sample into the reaction flask. The sample was purged by bubbling 50 percent N_2 through the solution. Five ml of 5 percent (w/v) sodium borohydrate was added through the septum while stirring.

Statistics

Basic parametric statistical tests (mean, standard deviation, standard error, 95 percent confidence intervals, correlation, two-factor ANOVA; Sokal and Rohlf, 1969) were carried out for this report. Statistical tests of data for 1974 through 1978 suggested that fluoride levels in bees may not be normally distributed (Bromenshenk, 1979, 1980). Therefore, the 1979 data will be re-examined via nonparametric tests such as Kruskal-Wallis and Wilcoxon two sample tests (Conover, 1971) in order to examine possible differences in the distributions of fluoride levels in bee populations. Also, the data obtained since 1974 indicates standard errors for fluoride and arsenic which are a constant function of the mean. Because of this, M. E. Ginevan, biomathematician and entomologist at Argonne National Laboratories (personal communication, 1980) recommends logarithmic transformations of the data prior to applying parametric statistics. Each of the above approaches will be undertaken, the results of which will be published in a final report covering the entire 6-year study period. It was not possible to complete these exercises for this progress report.

RESULTS

Fluoride levels in apiary water supplies are summarized in Appendix 20.1. Rosebud Creek, which supplies water to 87 percent of the beeyards near Colstrip, displayed relatively constant fluoride levels from 1974 through 1979

(0.4-0.6 ppm). Water from deep wells (NE 10 and E 2) contained more fluoride (2.0-8.0 ppm) and a reservoir (S 1) had the least fluoride (0.1 ppm). A livestock watering tank and a slough formed by overflow from the tank located at NE 10 has had the highest fluoride levels (2.6-12.7 ppm). Although bees have often been observed obtaining water at Rosebud Creek and at the stock tank and slough at NE 10, bees were not seen at the stock tank at E 2 and appeared to be using Rosebud Creek as their primary water supply.

Fluoride levels in pollen obtained from each beeyard are presented in Table 20.1. From 1975 through 1978, mean fluoride levels of pollen obtained from beeyards did not exceed 3.0 ppm (range 1.5-2.9 ppm). In 1979, 3.0 ppm was exceeded in 43 percent of all cases (June/July and September) and 57 percent of the June/July samples; 4.3 ppm at NE 3 in June was the high. Mean fluoride in excess of 3.0 ppm was found in two check samples (3.4 ppm at SE 6 in September, 3.1 ppm at SE 12 in June/July) as well as at sites near Colstrip.

TABLE 20.1. PPM FLUORIDE IN POLLEN, 1979

Site	Date	$\bar{X}$	S.D.	S.E.	N
N 1	Sept.	1.5	0.69	0.23	9
NE 1	June/July	1.9	0.56	0.18	10
	Sept.	2.9	0.72	0.28	10
NE 2	June/July	2.0	0.57	0.18	10
	Sept.	1.8	0.69	0.22	10
NE 3	June/July	4.3	0.92	0.29	10
NE 4	June/July	3.1	1.43	0.54	7
NE 3/4	Sept.	3.6	1.04	0.29	13
E 2	June/July	2.7	1.04	0.33	10
	Sept.	2.4	0.84	0.26	10
SE 1	June/July	3.8	0.39	0.12	10
	Sept.	4.0	0.85	0.27	10
S 4	June/July	3.2	1.06	0.35	10
	Sept.	3.4	0.75	0.24	10
S 5	June/July	2.5	1.07	0.34	10
	Sept.	2.4	0.74	0.23	10
S 1	June/July	3.2	1.13	0.36	10
	Sept.	2.7	0.96	0.30	10
SW 2	June/July	2.2	0.42	0.13	10
	Sept.	2.6	0.91	0.29	10
SW 3	June/July	3.2	1.25	0.40	10
	Sept.	2.0	0.55	0.17	10
SE 6	June/July	2.7	1.01	0.34	10
	Sept.	3.4	1.00	0.32	10
SE 12	June/July	3.1	0.78	0.25	10
	Sept.	1.6	0.24	0.07	10
NE 10	June/July	2.8	0.61	0.20	10
	Sept.	3.8	0.51	0.16	10

Mean content and 95 percent confidence intervals for fluoride in worker honey bees collected in mid-summer and autumn of 1979 are presented in Figures 20.1 and 20.2 and Table 20.2. Figure 20.1 also presents a map of the major apiary locations utilized since 1974. Figure 20.3 presents mean fluoride content and 95% confidence intervals for samples taken in autumn of 1975, 1976, 1977, and 1978.

Mean fluoride levels of pollen collected from each apiary in June and September, 1979, were not significantly correlated ($r = 0.20$, df 12, $P \geq 0.05$), nor did mean fluoride content of bees collected at beeyards in September significantly correlate with mean fluoride content of pollen collected in September ($r = 0.31$, df 12, $P \geq 0.05$). There was a significant, although weak, correlation between fluoride levels in pollen and bees from each site (mean fluoride) and from each hive ($r = 0.59$, df 11, $P \leq 0.05$; $r = 0.3721$, df 126, $P \leq 0.01$) based on June/July, 1979, sampling results.

The absolute mean fluoride content of honey bees sampled in June/July of 1979 at all sites except NE 10 exceeded that of the previous 2 years (Figure 20.1). A livestock watering tank has been a potential source of fluoride intake by bees at NE 10 since 1974 (Bromenshenk, 1978, 1979, 1980). It is impossible to make meaningful comparisons of 1979 to 1976 data for the July period. A breakdown of the electric vacuum sampler in 1976 necessitated taking bees by sweeping them from honeycombs rather than by catching them at hive entrances. Tests conducted in September of 1976 indicated that bees taken from inside hives contained 50 percent less fluoride than bees collected at entrances (Bromenshenk, 1978). However, even doubling the levels of fluoride reported for July of 1976 would still result in a mean of less than 12 ppm for Colstrip bees at all sites. Bees collected from 17 sites in 1975 displayed a mean fluoride content of 8.7 ppm, SE = 0.44, as compared to 27.8 ppm, SE = 8.87, for 14 sites in the same area in 1979.

Site NE 4 had a mean fluoride content of 30.9 ppm in early summer of 1979. A mean of 153.8 ppm fluoride in bees at NE 3 in June was the highest value observed at any apiary during the 6 years of this project. Mean values of 53.2 ppm at S 1 in 1979 and 35 ppm in 1977 greatly exceeded the 4.9 ppm for 1978 and 5.2 ppm for 1975. In general, fluoride levels in bees at all of the sites near Colstrip, but not at the "check" sites (SE 6 and SE 12), ranked among the highest observed in this region.

Although water contributes fluoride to bees at NE 10, water at NE 4, NE 3, and S 1 is unlikely to be the source of the fluoride in bees. The reservoir at S 1 typically contains not more than 0.1 ppm fluoride (1976 through 1979 data), while NE 2 and NE 3 are supplied by water from Rosebud Creek (0.4-0.6 ppm), as are most of the other Colstrip beeyards.

Bees sampled in September of 1979 most closely resembled those of 1976, both in terms of mean fluoride content and the geographical distribution of "elevated" levels of fluoride. The use of two-factor ANOVA demonstrated highly significant year and site differences among variances for 1979 versus 1975 levels of fluoride in autumn samples of worker honey bees:

$F^1_s = 12.73 > F_{.001[1,22]} = 6.73$ (years); $F^2_s = 9.20 > F_{.001[24,160]} = 2.13$ (sites)

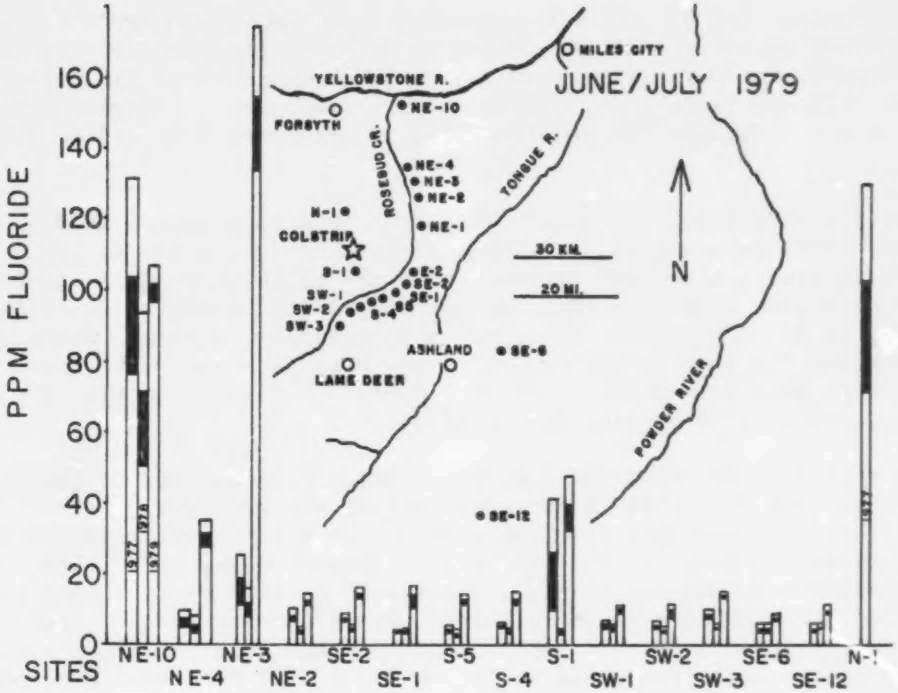

Figure 20.1. Mean fluoride in worker honey bees and 95 percent confidence intervals, June/July collections 1977, 1978 and 1979.

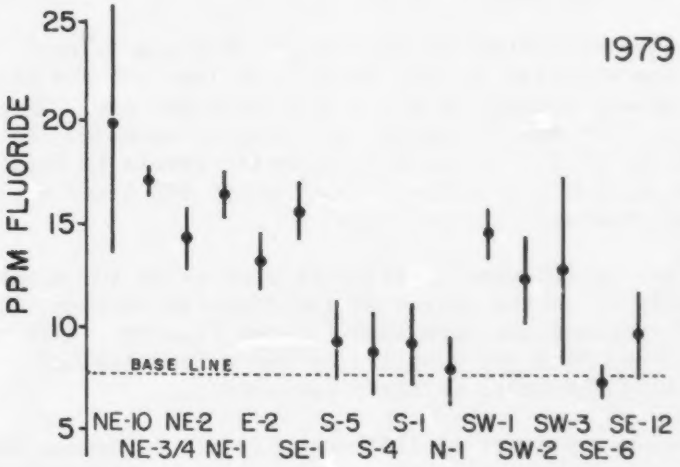

Figure 20.2. Mean fluoride content and 95 percent confidence intervals of worker honey bees collected in autumn, 1979. Site E 2 was utilized in 1979 in lieu of SE 2. It falls within the forage area (flight range) and has the same water supply as SE 2.

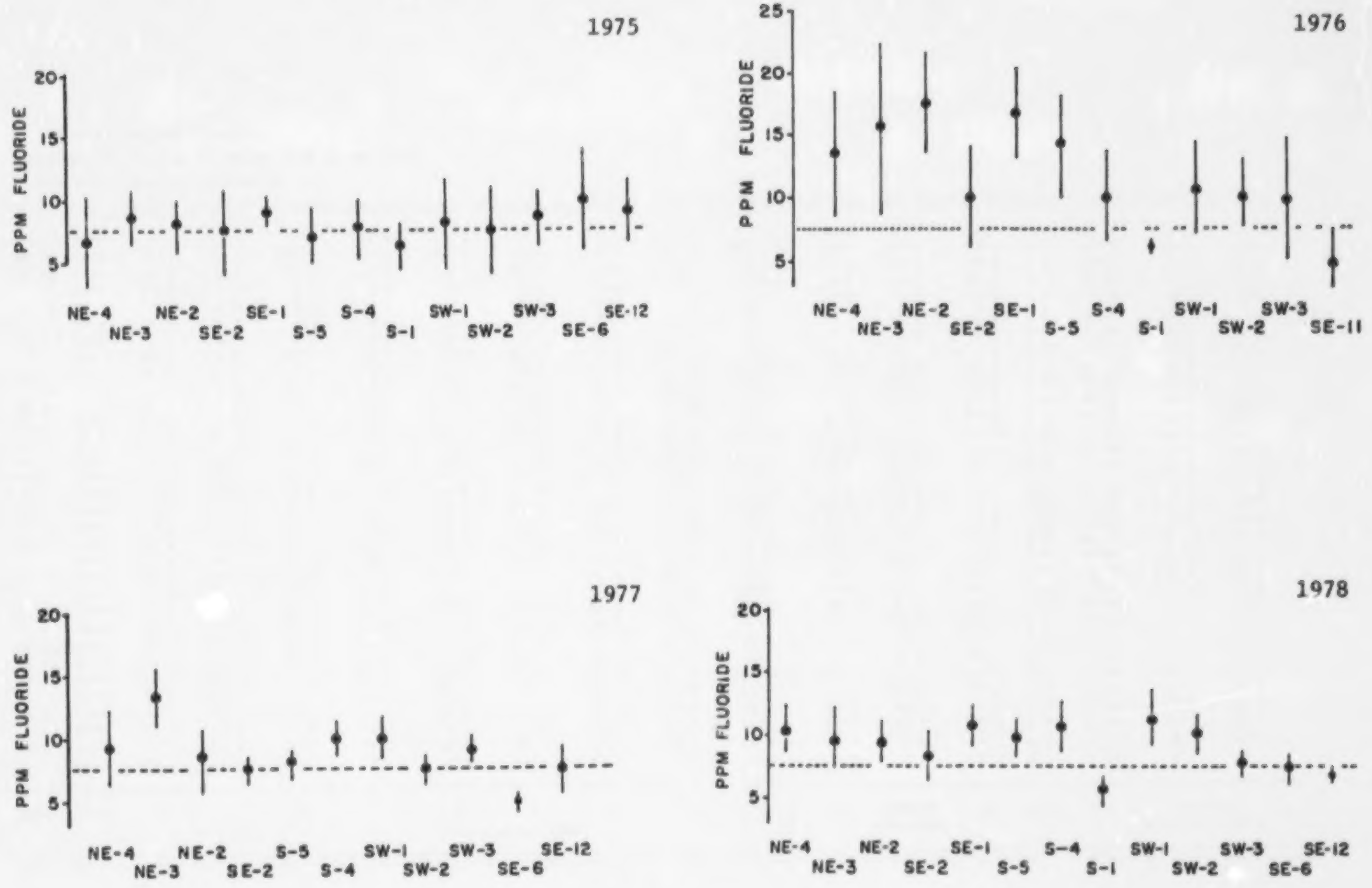

Figure 20.3. Mean fluoride content and 95 percent confidence intervals of worker honey bees collected in autumn, 1975, 1976, 1977 and 1978. Due to occurrences such as grass fires, lack of forage, and dry ponds, not all sites were utilized over all years.

TABLE 20.2. FLUORIDE CONTENT OF ADULT WORKER HONEY BEES, 1979

Site	Date	Sample Hives (ppm)										Combined Sample	$\bar{X}$	S.D.	S.E.	95% Confidence Interval
		1	2	3	4	5	6	7	8	9	10					
N 1	Sept	10.5	11.0	10.7	5.7	6.1	7.7	10.5	5.4	6.4	6.4	8.1	8.0	2.3	0.74	6.4 - 9.7
NE 1	June	23.0	23.2	20.6	21.2	30.6	28.3	15.4	18.7	28.4	25.3	23.7	23.5	4.8	1.50	20.1 - 76.9
	Sept	15.7	15.3	15.6	15.2	19.3	15.3	15.2	18.4	17.9	15.2	17.7	16.3	1.6	0.50	15.2 - 17.4
NE 2	June	18.6	12.7	16.2	17.6	19.9	15.7	14.2	16.8	18.9	16.8	15.0	16.7	2.2	0.69	15.2 - 18.3
	Sept	14.2	14.3	13.0	16.1	14.5	16.1	19.7	12.4	13.4	11.8	16.5	14.6	2.3	0.73	12.9 - 16.0
NE 3	June	175.2	171.8	135.5	115.9	137.6	190.9	182.2	109.4	150.5	169.2	133.2	153.8	28.3	8.96	133.6 - 174.1
NE 4*	June	32.4	28.8	34.6	22.6	33.4	32.8	32.0	-	-	-	34.9	30.9	4.1	1.29	27.2 - 34.7
NE 3/4	Sept	21.3	15.3	20.2	17.1	19.7	14.1	17.7	16.1	18.3	17.8	-	-	-	-	---
	Sept	16.1	16.9	17.2	15.9	15.9	17.4	16.9	18.7	16.5	-	18.9	17.3	1.8	0.57	16.5 - 18.2
E 2†	June	22.0	18.1	16.1	22.2	23.0	24.0	19.5	16.9	17.0	20.5	19.1	19.9	2.8	0.88	17.9 - 21.9
	Sept	10.8	16.7	11.6	11.3	12.9	13.4	13.7	13.5	13.8	16.8	13.1	13.4	2.0	0.64	11.9 - 14.9
SE 1	June	20.5	24.1	18.6	13.2	16.1	15.0	25.0	15.5	25.7	19.1	18.6	19.3	4.4	1.41	16.1 - 22.5
	Sept	16.9	18.7	16.3	18.4	16.6	13.9	13.8	16.8	12.6	15.2	16.4	15.9	2.0	0.64	14.5 - 17.4
S 4	June	14.8	20.0	16.1	15.0	13.2	20.6	15.4	18.6	18.1	20.2	16.1	17.2	2.6	0.76	15.3 - 19.1
	Sept	6.8	10.2	7.7	11.0	6.7	8.0	4.9	7.8	12.8	13.4	13.0	8.9	2.8	0.88	6.9 - 10.9
S 5	June	14.2	17.3	15.7	19.2	20.2	15.9	15.9	15.3	12.4	20.8	14.9	16.7	2.7	0.85	14.8 - 18.6
	Sept	9.6	13.0	5.6	11.0	7.6	7.9	-	13.4	7.2	9.5	10.8	9.4	2.7	0.84	7.5 - 11.3
S 1	June	43.7	63.5	53.8	70.0	39.5	31.7	49.0	78.1	41.5	61.5	59.5	53.2	14.8	4.68	42.6 - 63.8
	Sept	11.1	8.0	11.2	10.7	6.8	7.5	7.6	12.7	7.7	7.3	10.9	9.1	2.1	0.66	7.5 - 10.6
SW 1	June	14.1	14.5	11.8	13.5	13.0	16.1	12.3	14.0	15.2	13.1	-	13.8	1.3	0.41	12.8 - 14.7
	Sept	14.0	14.3	13.3	15.0	13.3	16.5	13.8	12.4	16.4	17.7	18.0	14.7	1.7	0.59	13.4 - 15.8
SW 2	June	10.1	16.2	17.7	12.0	12.8	7.9	13.2	14.5	12.1	11.9	12.2	12.8	2.8	0.89	10.8 - 14.8
	Sept	11.0	12.9	12.1	13.9	12.9	10.3	11.1	8.0	12.9	19.3	15.0	12.4	2.9	0.93	10.3 - 14.6
SW 3	June	14.1	14.5	11.8	13.5	13.0	16.1	12.3	14.0	15.2	13.1	15.0	13.7	1.3	0.41	12.8 - 14.7
	Sept	12.3	7.9	10.5	12.1	12.3	12.1	9.7	13.2	9.0	30.6	11.7	13.0	6.4	2.02	8.4 - 17.6
SE 6	June	7.1	5.4	6.5	8.5	7.0	7.2	6.5	5.2	7.2	8.4	6.3	6.9	1.1	0.35	6.2 - 7.7
	Sept	7.5	7.1	8.3	8.5	7.7	6.9	8.4	5.3	6.6	8.8	10.3	7.5	1.1	0.34	6.7 - 8.3
SE 12	June	11.1	10.8	7.7	9.1	7.3	11.9	9.8	13.1	9.3	12.3	7.0	10.2	1.9	0.30	8.9 - 11.6
	Sept	13.0	6.6	7.6	7.8	9.0	9.8	15.4	10.9	11.9	6.7	14.0	10.0	3.1	0.96	7.8 - 12.2
GB 3‡	Sept	10.8	8.3	11.8	11.7	12.9	13.2	11.5	13.4	14.8	13.8	14.3	12.2	1.8	0.58	10.9 - 13.5
NE 10	June	97.8	101.3	116.4	104.5	100.2	94.6	101.0	89.1	107.0	98.7	85.4	85.4	7.3	2.31	80.2 - 90.6
	Sept	36.3	25.3	14.4	14.9	7.9	33.2	32.7	14.8	16.4	15.0	32.1	32.1	9.9	3.13	39.2 - 19.80

* Only seven colonies in beeyard.

† Located north of SE 2, within same forage area.

‡ Located at Billings, Montana.

Two-factor ANOVA for 1979 versus 1976 levels of fluoride in bees from August/September revealed highly significant differences in variances among sites but not years:

$$F_s^1 = 1.11 < F_{.05[1,23]} = 4.28 \text{ (years)}; \; F_s^2 = 11.14 > F_{.001[20,144]} = 2.51 \text{ (sites)}$$

Comparing absolute mean fluoride content of bees (95 percent confidence intervals) for autumn of 1975 through 1979, levels in 1979 and 1976 were generally higher than in 1975, 1977, and 1978.

In both 1979 and 1976, fluoride levels were lowest at sites directly south of Colstrip and at the "checks" distant from Colstrip. The 1979 mean fluoride content of bees samples in September exceeded 1975 baselines and equalled or exceeded levels observed in 1977 and 1978 at sites northeast, east, southeast, and southwest of Colstrip (Figure 20.3). The 1979 fluoride levels in bees collected in September were lower than those of June/July at many Colstrip sites, although fluoride levels at apiaries southwest of Colstrip and at the check sites were essentially the same for both the early and late summer periods.

Fluoride Values for Pooled Samples Versus Mean of Separate Samples

Values for fluoride in pooled samples versus the mean of independent samples are presented in Figures 20.4 and 20.5. Figure 20.4 summarizes data from all sites from 1974 through 1979. The correlation coefficient ($r = 0.99$) indicates a highly significant relationship, $P \leq 0.001$. The coefficient of determination ($r^2 = 0.98$) indicates that only slightly over 2 percent of variation in fluoride indicated by the mean of independent observations is due to variation not associated with "fluoride content" as displayed by the pooled samples.

Figure 20.5 shows cases in which fluoride content was less than 20 ppm. Here $r^2 = 0.80$ signifies that 80 percent of fluoride variation in the mean of independent observations is associated with fluoride, as indicated by pooled samples. Again, the association is significant; less than 3 percent of observations would be expected to fall outside the 3σ confidence intervals. Figure 20.5 includes mean values based on as few as four independent colonies. I recomputed the correlation coefficient and coefficient of determination for only those values based on eight to 10 observations and obtained values of $r = 0.94$, $r^2 = 0.883$.

Arsenic

The results of 63 arsenic determinations demonstrated levels equivalent to those of baseline at all sites during 1979. The highest recorded value was 0.51 ppm at E 2 in September; the lowest value was 0.13 ppm at SE 12 in September. The data indicated somewhat lower values in bee samples ground in a Wiley Mill® before analysis, compared with the levels in whole bees. It was concluded by the chemist performing the analyses that the ground tissues remained damper than the whole bees after oven-drying.

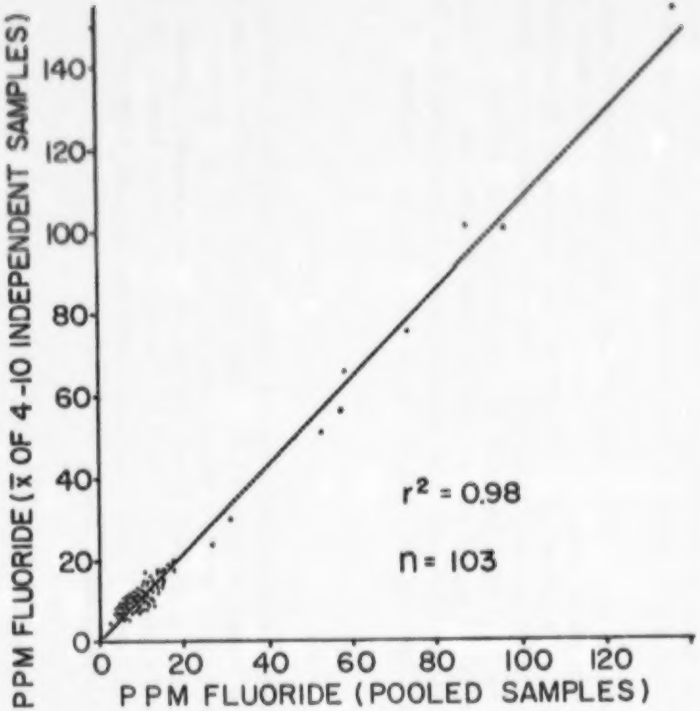

Figure 20.4. Comparison of fluoride content of worker honey bees as determined by pooled samples and by the mean of independent observations over a 5 year period.

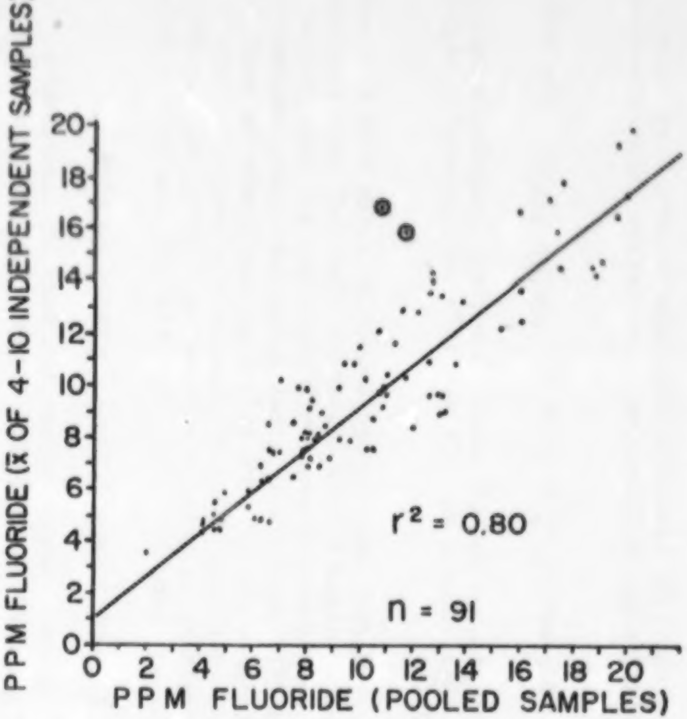

Figure 20.5. Comparison of fluoride content of worker honey bees as determined by pooled samples and by the mean of independent observations over a 5 year period for values less than 20 ppm. The circled values were based on four observations.

DISCUSSION

Fluoride and arsenic are released by coal-fired power plants. Measured stack concentrations of Colstrip Unit 2 indicate a level of 2,130 ± 400 (SD) ppm for fluoride and 221 ± 20 (SD) ppm arsenic (Crecelius *et al.*, 1978). Munshower (personal communication, 1978) has detected increased postoperational arsenic levels in pine needle sheaths from trees near Colstrip. His analysis of 1977 bee samples showed arsenic levels in bees from NE 2 were 2 to 3 times higher than baseline and check site levels. Other investigations have shown substantially higher fluoride levels in mice and pine foliage from sites near Colstrip compared to more distant sites (Gordon *et al.*, 1978, 1979). My own studies have repeatedly demonstrated significant postoperational fluoride changes in bees from apiaries downwind and as far as 20 km from Colstrip (Bromenshenk, 1976, 1978, 1979, 1980).

Fluoride in honey bees was used in this study as a tracer in an attempt to determine the distribution patterns of the power plant plumes. The 1979 fluoride data in bees is consistent with previously reported wind patterns (Van Valin *et al.*, 1980; Ludwick *et al.*, 1980; Bromenshenk, 1979).

Although summer winds are usually westerly during the daytime, night surface winds are variable and typically light. Furthermore, the plumes from the power plants are affected by the underlying terrain topography being diverted from the direction of the prevailing winds by as much as 20°. Plumes follow valleys and are deflected around higher terrain (Van Valin *et al.*, 1980). It appears that plumes may be trapped in valleys and then flow southward with northerly winds mixing at times to ground levels (Ludwick *et al.*, 1980). Plumes have been tracked as far as 50 km from Colstrip. Therefore, although several years of wind tower data gathered by the Montana State Department of Health suggest that prevailing winds in order of importance are E-SE, W-NW, and E-NE, this may be only a crude approximation of plume dispersion patterns. The data obtained from the sulfation and formate plates which were set out in 1978 and 1979 at each apiary should prove useful in determining whether fluoride is reaching the apiaries via the air. Unfortunately, the analytical results were not returned in time for incorporation into this report.

As in previous years, the highest fluoride levels in bees in autumn of 1979 were at apiaries located in a "downwind" or easterly direction from Colstrip (NE, SE, E). Apiaries directly south and north of Colstrip and the more distant checks exhibited levels essentially identical to baselines. Also, as in other years, sites located just west of directly south displayed intermediate levels of fluoride somewhat higher than baselines. The results from autumn of 1979, both as regards levels and the geographic distribution of fluoride in bees, were similar to those of 1976.

At all locations including the checks, the June/July fluoride content of bees was as high or higher than those observed in 1977 and 1978. A prolific flowering of yellow sweet clover in June provided plentiful supplies of nectar and undoubtedly stimulated foraging activity. This may have contributed to the generally higher fluoride levels because of more flight activity, greater probability of contact, more materials brought back to the hives, etc. However, mean fluoride content of bees obtained at each of two "check" apiaries in 1979 (approximately 40 and 80 km from Colstrip) was lower than that of the bees from the Colstrip locations.

Disregarding NE 10, the highest fluoride concentrations for 1979 occurred at NE 3, NE 4, and S 1 during June and July. Only once before were levels greater than 30 ppm observed--at sites N 1 and S 1 in June/July, 1977. Dumping mine waste waters into Arnell's Creek (the apiary's water supply) may have caused high levels of fluoride in bees at N 1 (Bromenshenk, 1980). No source of the fluoride at the other sites is known. It was not in any of the water supplies sampled. These fluoride levels are cause for concern. Levels observed at NE 3 could poison bees based on literature reports and my own observations (Bromenshenk, 1980).

Long-term studies are needed to determine how serious these fluoride levels in bee systems may be and how the bees are taking up the fluoride. The weak correlation of fluoride levels in bees and pollen collected in June/July suggests the airborne fluoride may be reaching the bees via the food. However, one would expect the highest levels to occur in nurse bees or pupae if bioaccumulation is via pollen and in foragers if from nectar. My previous studies (Bromenshenk, 1978, 1979, 1980) showed little if any fluoride accumulation in pupae and hive bees (mainly nurse bees) and very low levels of fluoride in floral parts, which provides indirect measure of fluoride in nectar. It is possible that the high fluoride concentrations observed in field bees came about as a result of exposure to airborne contaminants either via penetration of the cuticle, which seems unlikely, or via the tracheal system. This is an attractive hypothesis since food correlations (pollen) are so low, $r^2 < 14$ percent.

However, there are possible explanations for such a weak correlation between levels in pollen and bees. P. Tourangeau, who carried out all of the fluoride analyses, suggested that the levels of fluoride in pollen are near the lower limits of detectability of the Orion probe, using our present methods, and the sensitivity may not be good enough in this range to reliably separate the signal from the noise. We currently are investigating this possible source of error.

There is always the possibility that the fluoride seen in early summer got into the bee systems before the colonies were set out in the Colstrip area. However, it is likely that the fluoride accumulation was actually caused by exposures in Montana. Any fluoride carried back in colonies exposed at apiaries in California during the winter should be diluted and "cleaned out" by June/July for the following reasons:

1. Preoperational studies did not detect any fluoride carried back from California.

2. Except for the brood boxes, none of the equipment is taken to California. Brood cells are lined by bees with a "papery" material which effectively isolates the brood from the wax.

3. Bees are returned to Montana with marginal food stores which are rapidly consumed.

4. The bees had been at the Colstrip locations since late April and early May which is sufficient time for replenishment of stores and for several population turnovers.

5. All of the sampled colonies had a considerable amount of surplus honey in the "honey supers" at the time of collection, indicating they had been at the beeyards long enough to build up food reserves.

6. Marked hives taken to California and located upon their return indicated that the boxes become well mixed and more or less randomized while being stockpiled, inspected for disease, split to form new colonies, and transported via truck.

Also, one would not expect to see all colonies within a given Colstrip apiary displaying similar levels of increased fluoride, *e.g.*, NE 3 samples were all greater than 100 ppm, those of S 1 were all greater than 31 ppm but less than 70 ppm, *etc.* Fluoride brought back from California should show up as a more random pattern--some colonies at a given location displaying high levels, others intermediate and some very low--since the colonies at any Colstrip beeyard probably came from several California beeyards.

As in all previous years, the fluoride in bees at NE 10 appeared to be associated with the fluoride in water in a nearby stock tank. These colonies were moved farther from the tank and closer to Rosebud Creek in 1979 in an attempt to change their water supply. Fluoride levels in these bees was still high in June/July but for the most part were below 100 ppm. They declined to less than 36 ppm by September. Although high, these levels, especially in September, are lower than those observed in individual colonies at this site during previous years and are below the 120-130 ppm levels, which seem to "definitely" indicate acute poisoning. The colonies at this apiary have always been characterized by a lack of vigor, poor brood laying, and low honey production. In 1979, the beekeeper reported that this was one of his best apiaries, producing more than twice as much honey per colony as in previous years. This suggests at least a partial solution to this specific problem and tends to confirm the assumption that fluoride levels exceeding 100 ppm were affecting these colonies. However, the data from 1979 indicate that merely moving the bees farther from a "contaminated" water supply and closer to a "clean" water supply does not guarantee that the bees will utilize the preferred one.

The fluoride levels in water at sites other than NE 10 were almost identical to previous levels, and water does not appear to be the source of the high fluoride levels in the bees during either of the 1979 sample periods. A stock watering tank about 0.3 km from E 2 contained more fluoride than Rosebud Creek, which was very close to the apiary. The fluoride concentration in bees at E 2 was higher in June/July than in September, but that of the water was considerably higher in September. Thus, it is improbable that the stock tank in this case was a major contributor of fluoride.

It is possible that fluroide in water may be a contributing factor. Many ranchers in the area have complained of wells going bad since the mining activities began. Also, mine waste waters apparently have sometimes been discharged into streams. However, most apiaries are located near Rosebud Creek and relatively distant from other water supplies; fluoride levels in the Rosebud have remained almost constant since 1974.

It is becoming apparent that fluoride either via air and/or water is reaching apiaries near Colstrip and at levels which may pose potential hazards to bees and beekeeping. The fluoride data from 1979 raises many questions and only suggests possible answers.

Although arsenic appeared at relatively high levels at an apiary northeast of Colstrip in 1977, this was not observed in 1979. If arsenic were to sporadically impact the apiaries, longer term monitoring would be needed to adequately address potential for buildup of this toxic material. One might expect to see correlations between levels of arsenic and fluoride in bees if these materials are being inserted into their environs by the combustion of coal or by coal-mining activities. The one instance of "elevated" arsenic in 1977 was at a site that over the years has tended to display higher fluoride levels. According to Crecelius *et al.* (1978), 10 times more fluoride than arsenic is emitted by the power plants. Thus, it is not surprising that arsenic levels in bees are low in comparison to fluoride. In addition, the two contaminants are dissimilar in physical and chemical aspects which may affect factors such as dispersion and transport in the plume, uptake routes into bee systems, chemical forms encountered, and biochemical/physical chemical interactions.

CONCLUSIONS

Honey bees collected in 1979 at apiaries within 20 km of Colstrip, Montana, failed to show any arsenic levels above baselines but continued to show significant postoperational fluoride changes compared with preoperational levels. Unusually high mean fluoride levels in bees, ranging from 2 to 17 times baselines, were found at several beeyards sampled in early summer. These levels in bees did not correlate with levels in water supplies. A significant correlation ($P \leq 0.05$) was obtained for fluoride in pollen and bees, although the correlation coefficient was weak ($r = 0.37$).

Bees and pollen from a site northeast of Colstrip in June of 1979 had the highest mean fluoride content (154 and 4.3 ppm, respectively) ever recorded from southeastern Montana. According to literature reports and my own observations, this level indicates poisoning. Levels greater than 30 ppm were observed at sites northeast and south of Colstrip during June/July, 1979. Very high fluoride values were observed in June/July of 1977 at two sites. One of these was S 1, which in 1979 had 1.5 times the "high level" of 1977 or 53 ppm versus 35 ppm; baselines averaged 5 ppm for this site.

Fluoride concentration in the autumn, 1979, collections did not demonstrate the unusually high levels of the earlier sample period, although mean fluoride was approximately double that of baselines at sites northeast and southeast of Colstrip and somewhat higher than baselines at sites southwest of Colstrip. The patterns of fluoride concentration and distribution were very similar to significant postoperational increases of fluorides in these bee systems in 1976. Fluoride concentrations in bees from the "check" sites approximated baselines, and levels in September were the same as those in July.

APPENDIX 20.1. PPM FLUORIDE IN APIARY WATER SUPPLIES, 1979

Dates	NE 10	NE 4	NE 3	NE 2	NE 1	N 1*	E 2†	E 2
June/ July	0.6	0.6	0.6	0.6	0.4	-	2.6	0.6
Sept.	0.5	0.4	0.6	0.6	-	0.4	6.3	0.6
	SE 1	S 5	S 4	S 1‡	SW 1	SW 2	SE 3	NE 10†
June/ July	0.6	0.6	0.6	0.1	0.4	0.4	0.6	8.3
Sept.	0.6	0.5	0.6	0.1	0.4	0.6	0.6	10.4

Rosebud Creek Sites (1977)	Rosebud Creek Sites (1978)	Rosebud Creek Sites (June/July 1979)	Rosebud Creek Sites (Sept. 1979)
$\overline{X}$ = 0.51	$\overline{X}$ = 0.55	$\overline{X}$ = 0.55	$\overline{X}$ = 0.54
S.D. = 0.03	S.D. = 0.10	S.D. = 0.09	S.D. = 0.08
S.E. = 0.01	S.E. = 0.03	S.E. = 0.03	S.E. = 0.03
N = 10	N = 10	N = 11	N = 10

* Arnell's Creek (a dry creek most of the summer).
† Livestock Watering Tank.
‡ Reservoir.

Regression analyses of the results of fluoride determinations for samples "pooled" at the time of collection versus the mean fluoride content of four to 10 "individual colonies" revealed significant, $P \leq 0.001$, correlations. Pooled samples appear to be adequate and reliable for use in a rapid screening procedure to locate "hot spots" of pollutant contamination. Observations of values for individual hives increases information content but may not be necessary for an initial monitoring effort.

The 1979 data raises critical questions, while only suggesting answers. It is apparent that fluoride is impacting apiaries near Colstrip at levels which may harm bees and beekeeping. Whether the fluoride is coming from air or water or both media is unclear. Patterns of fluoride in pollen and the geographical distribution of fluoride buildup with respect to Colstrip and the prevailing winds suggest airborne fluoride.

REFERENCES

Bromenshenk, J. J. 1976. Investigations of the Effects of Coal-Fired Power Plant Emissions Upon Insects, Report of Progress. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana. R. A. Lewis, N. R. Glass, and A. S. Lefohn, eds. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 112-129 and 286-312.

Bromenshenk, J. J. 1978. Investigations of the Impact of Coal-Fired Power Plant Emissions Upon Insects. I. Entomological Studies in the Vicinity of Colstrip, Montana. II. Entomological Studies at the Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana. E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 146-312 and 473-507.

Bromenshenk, J. J. 1979. Honeybees and Other Insects as Indicators of Pollution Impact from the Colstrip Power Plants. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 215-239.

Bromenshenk, J. J. 1980. Accumulation and Transfer of Fluoride and Other Trace Elements in Honeybees Near the Colstrip Power Plants. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn, eds. EPA-600/3-80-052, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 72-95.

Conover, W. J. 1971. Practical Nonparametric Statistics. John Wiley and Sons, New York, New York. 462 pp.

Crecelius, E. A., L. A. Rancitelli, and S. Garcia. 1978. Power Plant Emissions and Air Quality. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems, ERDA Annual Report. Activity RS-02-03, Ames Laboratory, Iowa State University, Ames, Iowa. pp. 9-33.

Gordon, C. C., P. C. Tourangeau, and P. M. Rice. 1978. Potential for Gaseous Contamination from Energy Extraction Processes in the Northern Great Plains. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems. ERDA Annual Report, Activity RX-02-03, Ames Laboratory, Iowa State University, Ames, Iowa. pp. 53-137.

Gordon, C. C., P. C. Tourangeau, and P. M. Rice. 1979. Foliar Pathologies of Ponderosa Pine Near Colstrip. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 141-214.

Hillmann, R. C. 1972. Biological Effects of Air Pollution on Insects, Emphasizing the Reactions of the Honeybees (*Apis mellifera* L.) to Sulfur Dioxide. Ph.D. Thesis, The Pennsylvania State University, University Park, Pennsylvania. 159 pp.

Lillie, R. J. 1972. Air Pollutants Affecting the Performance of Domestic Animals--A Literature Review. U.S.D.A. Agriculture Handbook No. 380. 109 pp.

Ludwick, J. D., D. B. Weber, K. B. Olsen, and S. R. Garcia. 1980. Air Quality Measurements in the Coal-Fired Power Plant Environment of Colstrip, Montana. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn, eds. EPA-600/3-80-052, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 1-19.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, San Francisco, California. 776 pp.

Van Valin, C. C., R. F. Pueschel, D. L. Wellman, and G. M. Williams. 1980. Plume and Aerosol Properties Near Colstrip. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fifth Interim Report, Colstrip, Montana. E. M. Preston and D. W. O'Guinn, eds. EPA-600/3-80-052, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 20-48.

SECTION 21

BASELINE HISTOLOGY OF SELECTED ORGANS OF THE DEER MOUSE, *PEROMYSCUS MANICULATUS*, IN ROSEBUD COUNTY, MONTANA

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ABSTRACT

The normal histology of selected organs of the deer mouse (*Peromyscus maniculatus*) is presented. We believe that these organs are especially sensitive to long-term insults by low levels of pollutants such as those produced by coal-fired power plants. We also suggest that abnormalities in their structure due to such stress will be readily perceived by gross and histological examination. The organs include the male accessory reproductive glands, ovary, uterus, vagina, adrenal gland, spleen, liver, and kidney. Descriptions of the testis, epididymis, and heart of the deer mouse appear in an earlier report (Lewis *et al.*, 1978).

INTRODUCTION

This portion of our investigation of the deer mouse (*Peromyscus maniculatus*) provides quantitative descriptive information concerning the normal histology (central tendency and variation) of selected organs. Anatomical and histological abnormalities may provide useful indicators of long-term pollution impacts. The organs studied include the male accessory reproductive glands, ovary, uterus, vagina, adrenal gland, spleen, liver, and kidney. A basic understanding of these structures will help us to assess or predict trends and impacts of pollutants from coal-fired power generation (Lewis *et al*., 1978; Lewis and Lewis, 1979). These data are, in any event, essential to the interpretation of impacts that may occur in the future.

MATERIALS AND METHODS

Mice were trapped in southeastern Montana and transported alive to our field laboratory (Lewis *et al.*, 1978) where they were sacrificed and dissected in random rotation. Organs were immediately placed in 10 percent buffered neutral formalin or Bouin's solution. They were later weighed and examined in the laboratory, and then dehydrated, embedded in paraffin, and sectioned for histological study. Representative sections were stained with haematoxylin and eosin and evaluated.

Accessory Sexual Glands

The growth and maintenance of the accessory sexual glands depend directly on androgen production by the testis and in some cases characterize a male's reproductive condition better than the testis itself (Ewel, 1972). Accordingly, they are useful indicators of general reproductive condition, and specific androgen production.

We examined the vesicular gland, coagulating gland, and ventral prostrate glands for general structure, size, and seasonal changes. In addition, we used an ocular micrometer to determine the average diameter of each gland in section. We also measured the maximum diameter of this fusiform-shaped vesicular gland. Average tubule diameter is based on 10 independent measurements of the width of each vesicular gland. Measurements of 10 seperate tubules (or acini) were made in the case of the ventral prostrate and coagulating glands.

Ovary

Examination of the ovary is probably the most reliable method of determining the reproductive condition and maturity of female deer mice. For example, mature females have ovaries that contain corpora lutea (endocrine glands that develop from ovulated follicles), but immature animals do not. The number of corpora lutea in the ovary is also a measure of fecundity (number of eggs ovulated) and may be used to determine the egg production of mice (Coutts and Rowlands, 1969). The number of sets of corpora lutea, number of degenerate (atretic) follicles, abundance and appearance of interstitial tissue (which produces steroidal hormones), and the number and size of ovarian follicles within the ovary provide information about previous and current reproductive activity. Since follicular development is regulated by gonadotrophins produced by the anterior pituitary gland, ovarian histology can also be used to assess pituitary function (*e.g.*, Clarke and Kennedy, 1967).

Fixed ovaries (prior to imbedding) were examined with a dissecting microscope for grossly visible follicles, corpora hemorrhagica (follicles that have just ovulated), and corpora lutea. The following criteria were used to distinguish among the three: 1. Corpora hemorrhagica.--Small, punctate blood spots on the surface of the ovary; 2. Corpora lutea.--Round protrusions of variable size (classified as small, medium, or large) on the surface of the ovary; always solid and curdlike in appearance; 3. Mature (Graafian) follicles.--Small (always!) round protrusions on the surface of the ovary;

hollow, and in this way different from small corpora lutea with which they might otherwise be confused--a liquid-filled center (antrum) is visible through the wall of the follicle.

Serial sections of at least half of each ovary were examined for the number and size of (1) follicles at various stages of development, (2) corpora hemorrhagica, (3) corpora lutea, (4) atresias, and (5) scara (corpora albicantial). We also noted the amount and functional state (based on histological considerations) of interstitial tissue.

Uterus and Vagina

Uterus and vagina are two of the major regions of the reproductive duct of female deer mice. The vagina is especially useful for assessing the reproductive condition of an animal because there are obvious changes in its structure at various stages of the cycle, during pregnancy, and during sexual development. In contrast, changes in the structure of the uterine horn are subtle and difficult to interpret. It is nevertheless very useful for identifying newly pregnant females or those whose reproductive tracts contain embryos (blastocysts) that have not yet implanted.

We examined the uterine horn and vagina for changes associated with the principal reproductive states of female deer mice--reproductive inactivity, stages of the cycle, pregnancy, parturition and the immediately postpartum state, and lactation. We concentrated on epithelium, glands in the uterine horn, contents and size of the lumen, and characteristics of the connective tissue (lamina propria) and muscle (tunica muscularis). In the uterine horn, we quantified the height, mitotic activity, and number of inflammatory cells within the endometrial epithelium; the number of uterine glands, their diameter, and contents; the vascularity and width of the lamina propria and the degree to which it was infiltrated with inflammatory cells; and the width of the tunica muscularis. These characteristics were measured with an ocular micrometer or evaluated on a scale of 0 (none present) to 5 (very many or very high concentrations present). Averages are based on at least 10 measurements of each structure.)

Adrenal Gland

The adrenal gland of the deer mouse, like that of mammals generally, consists of two distinct and functionally independent glands: an inner medulla which produces catecholamines *(e.g.,* epinephrine), and an outer cortex which produces steroidal hormones *(e.g.,* corticosterone). Both parts respond to internal and external stressors. Epinephrine, for example, prepares the body to deal with immediate emergencies, increasing respiratory and cardiovascular activity, elevating blood sugar, and redirecting blood flow. As a biomonitor, the adrenal gland has the advantage of high sensitivity to external stressors. There is the further advantage that this gland responds nonspecifically to stress and therefore integrates all sources. The condition of the adrenal gland thus provides a basis not only for evaluating the degree of stress chronically recently experienced, but also provides information regarding tolerance or potential resilience to additional insults.

Its involvement with reproduction and certain other activities (see below) may complicate structural interpretation. However, knowledge of its normal structure in field populations of deer mice is essential to any evaluation of changes that may occur at sites of coal combustion.

To assess adrenal activity, we measured the cross-sectional areas of its major regions and the lipid content of the cortex. We also noted degenerative and pathological changes.

To determine cross-sectional areas of regions of the gland, we treated them as a series of concentrically arranged ellipsoids in cross-section. Four such ellipsoids exist in the normal gland. The innermost is the medulla. This is surrounded by the three major regions of the adrenal cortex: the zona reticularis (ZR), zona fasciculata (ZF), and zona glomerulosa (ZG), in that order. Accordingly, the other three ellipsoids are combinations of (1) medulla and surrounding ZF, (2) medulla, ZR, and surrounding ZF, and (3) medulla, ZR, ZF, and surrounding ZG. Because the area of ellipsoid is $\pi ab/4$, where a and b are its major and minor diameters, respectively, cross-sectional areas of each ellipsoid were readily obtained from measurements made with an ocular micrometer. However, it was necessary to combine the ZR and ZF for analysis because the boundary between them was frequently indistinct.

Mice that are either immature or have never been pregnant sometimes have additional cortical zones with unknown functions (Howard, 1927; Jones, 1957; Delost and Delost, 1954; Quay, 1960; Christian and Davis, 1964; Tähkä, 1979). Having only two immature animals, we did not include this zone in our analysis. It is thus possible that some areas classified as ZR in females whose reproductive condition was not accurately known are in error in this regard.

We also assumed that the sections of adrenal gland examined were from the center of each gland. Actual variation in the total cross-sectional areas of all glands examined supports this assumption.

The lipid content and the presence of degenerative areas in the adrenal cortex have been frequently used as indicators of adrenal activity (*e.g.*, Andersen and Kennedy, 1932; Allen, 1960; Dawson *et al.*, 1961; Christian and Davis, 1964). When the gland is functioning at "normal" levels of activity, there is a large amount of cortical lipid in the form of large droplets; at higher levels of activity (*e.g.*, under conditions of moderate stess), the amount of lipid diminishes and only small droplets may be seen. When the gland is overtaxed, cortical lipid may be absent, and degenerative areas are seen.

Although the tissues were not prepared by procedures designed specifically to preserve lipids, we attempted to roughly quantify the lipid in the cortex and the form in which it occurred. Observed areas of degeneration do not appear to be fixation artifacts.

Data on male and female deer mice were treated separately because estrogens promote cortical growth in small mammals, whereas androgens retard cortical development. Hence, the adrenals of female mice tend to be larger than those of males. We also grouped females according to reproductive state (immature, reproductively inactive, cycling, pregnant, or lactating) because the cortex exhibits periodic fluctuations in size and activity associated with reproductive condition. The cortex is large and highly active during estrus and lactation, but smaller and less active during diestrus and pregnancy (Andersen and Kennedy; 1932, 1933)

Social and population characteristics also influence the adrenal structure of rodents. Dominant individuals have smaller glands than subordinates; mice in small populations have smaller adrenals than those in larger populations (Christian, 1955; Andrews, 1970). These sources of variation are probably small in deer mice because of their relatively nonaggressive nature (Christian and Davis, 1964).

Spleen

The mammalian spleen has many functions, including production of red and white blood cells and platelets; destruction of old or damaged red cells; storage of blood; production of antibodies; and the removal of foreign bodies from the body fluids that circulate through it. It can thus be expected to detoxify or in some cases accumulate pollutants from coal combusion that occur systemically.

Our measurements in the spleen were designed to estimate its role in the production and/or storage of blood cells and platelets, destruction of erthrocytes, immune responses, and the removal of foreign materials from the body fluids. We suggest that this can be ascertained by measuring changes in absolute and relative numbers of the various cell types within the spleen. Accordingly, we identified 40 cells in the red pulp of each spleen by use of a reticule with 0.1-mm divisions. More specifically, we identified the cell at each 0.1-mm mark along the reticule in four areas of red pulp selected randomly.

We also determined: (1) The relative abundance of red and white blood cells after scanning the entire section at low magnification. Each spleen was assigned to one of five categories--red cells far less, less, equally, more, or far more numerous than white cells; (2) The amount of hemosiderin present, determined by scanning the section and then rating the concentration of pigment on a scale of 0 (none) to 5 (extremely high); (3) The number of germinal centers in the white pulp. We counted the number present and noted the phagocytic and mitotic activity within each; (4) The number of megakaryocytes and hemocytoblasts in section. In many cases we counted the number of such cells in the section. Since this number depends somewhat upon the area of the section, we also estimated the abundance of each on a scale of 0 (none) to 5 (very numerous).

Our rationale for using the above as measures of splenic activity are:

1. The relative and absolute abundance of megakaryocytes are estimators of the organ's role in platelet formation since megakaryocytes produce platelets.

2. The relative abundance of red and white blood cells and of erythrocytes, normoblasts, and hemocytoblasts in the red pulp are estimators of the

organ's role in the formation and/or storage of erythrocytes. (Hemocytoblasts and normoblasts are immature stages of erythrocytes).

3. The hemosiderin content of the spleen is a measure of the organ's role in the destruction of erythrocytes.

4. The relative abundance of red and white blood cells and of medium- and large-size lymphocytes, hemocytoblasts, myelocytes and metamyelocytes in the red pulp are estimators of the organ's role in white cell production. (Lymphocytes of these sizes, hemocytoblasts, myelocytes and metamyelocytes are immature white cells).

5. The number, size, and mitotic activity of germinal centers in the white pulp of the spleen reflect its role in antibody production.

6. The relative abundance of macrophages, plasma cells, medium- and large-sized lymphocytes, and neutrophils in the red pulp indicate how much foreign material is removed from the body fluids as they perfuse the spleen, since the recruitment of these cells is induced by such material. (Macrophages and neutrophils phagocytize foreign materials; plasma cells produce antibodies; lymphocytes produce antibodies and cytotoxic substances that destroy foreign bodies on contact).

Liver

The vertebrate liver has many functions. For example, it produces bile; stores and/or synthesizes lipids, glycogen and plasma proteins; and stores vitamins and minerals. It also detoxifies or removes from the blood numerous foreign and endogenous substances including organic pesticides, poisons, hormones and ammonia. It is thus very likely to be directly affected by air pollutants and to mediate many of the animal's specific responses to pollutant stress. Knowledge of the normal structure of this organ in deer mice is probably essential to the assessment of pollutant-related changes that may develop at sites of coal-fired power generation.

We thus examined the liver to determine its normal structure and to identify seasonal changes, particularly in the incidence of: (1) autolysis of hepatic tissue accompanied or not by cirrhosis; (2) glycogen depletion of liver cells (hepatocytes); (3) fatty degeneration of hepatocytes; (4) fat storage in hepatocytes; (5) foreign materials and pigment in Küpffer cells; (6) invasion of hepatic parenchyma by foreign bodies and/or inflammatory cells.

For purposes of this study, we used the following criteria to distinguish among glycogen depletion, autolysis and fatty degeneration: (1) Glycogen depletion--Hepatocytes more or less empty and unstained; only the cytoplasm surrounding the nucleus and along the margins of the cell is stained (in contrast to normal cells in which the cytoplasm is uniformly sprinkled with well-stained granules); (2) Autolysis--Hepatocytes as above, but also with pycnotic nuclei; (3) Fatty degeneration--Hepatocytes with unstained cytoplasmic vacuoles of variable size (usually small).

Mild autolysis (with little nuclear change) could not be distinguished from glycogen depletion. Such livers were arbitrarily placed in the latter category.

The above items, together with hepatic blood flow, were each rated on a scale of 0 (none) to 5 (heavy or pronounced). The validity of these measurements depends critically on rapid fixation. Tissue was thus invariably placed in the fixative within a few minutes following sacrifice of each specimen.

Kidney

The kidney (in addition to the liver and spleen) is a principal site of detoxification in the vertebrate, and thus is likely to be directly affected by inverse pollutants.

We examined the kidney primarily to determine its normal structure and associated pathologies. Examples of the latter are precipitated protein or concretions in the nephrons, dilatation of the nephrons, changes in their epithelial lining, degeneration of renal parenchyma, and the presence of hemosiderin, ascites fluid, inflammatory cells, or foreign bodies in the renal tissue.

RESULTS AND DISCUSSION

Accessory Sexual Glands of Male Deer Mice

Histology

The histology of the accessory sexual glands of several murine rodents (*e.g.*, house mice, voles, and to a limited extent, *Peromyscus*) has been described (Snell, 1941; Anthony, 1953; Lecyk, 1962; Arata, 1964; Clarke and Forsyth, 1964; Hrabé, 1970; Ewel, 1972). Our observations are generally similar to those presented in these earlier studies. Major differences among the glands occur in the epithelium and in the characteristics of the secretion. All have a wall that consists of a mucosal epithelium that borders the gland's lumen, an underlying connective tissue (the lamina propria), beyond this a tunica muscularis or coat of smooth muscle, and finally an outer serosal covering.

Vesicular Gland (Seminal Vesicle).--The mucosal epithelium of the active vesicular gland is a high, crowded, simple columnar layer. The cells are uniformly basophilic with a basal, vesicular nucleus. They contain a supranuclear vacuole of approximately nuclear size. This vacuole contains one to few prominent, large, deeply stained granules that resemble those in the gland's lumen. Many cells can be found discharging these granules into the lumen. Such supranuclear vacuoles do not occur in the other accessory glands.

The mucosa is thrown up into primary folds of variable length. They are frequently very long and narrow, consisting of little more than two layers of epithelial cells situated back to back. Secondary folds occasionally occur. Primary and secondary folds sometimes intersect forming a reticulum of epithelial-lined pockets near the margins of the gland's lumen.

The tunica muscularis is thick and heavy in the active vesicular gland, especially in comparison with the muscle layer of the other accessory sexual glands. In many places, it appears to be a single, longitudinal layer. Elsewhere, it consists of an inner circular and outer longitudinal or oblique layer.

This is the largest accessory sexual gland. It is comprised of completely or partially separated, adjoining compartments. These are separated from each other by inward extensions of the mucosa and underlying muscularis. The lumen is capacious and characteristically filled with intensely eosinophilic (bright red) secretion in preparations stained with haematoxylin and eosin. The secretion is homogenous or granular. So much is present that it fills each chamber and exhibits fractures.

When inactive, the gland is small with a slit-like empty lumen which is lined by an inactive, low columnar epithelium. Epithelial cells are mostly filled with the nucleus and exhibit vacuolar degeneration. Mucosal folds still penetrate the lumen and compartmentalize the gland, and the reticulum formed by intersecting primary and secondary folds may fill the entire lumen. The lamina propria is densely cellular connective tissue, relatively wider than that of the active gland. Two layers of highly cellular and dedifferentiated smooth muscle form the tunica muscularis.

Coagulating Gland (Anterior Prostate Gland)--This compound tubular gland nests in the lesser curvature of the vesicular gland and is somewhat smaller than the latter. When active, its lumen is lined by a simple cuboidal to columnar layer consisting of cells with well defined cell membranes, basal vesicular nuclei, and much apical cytoplasm filled with fine eosinophilic granules. The epithelial cells are brick red, in contrast to those of all other accessory glands. Each cell is distinctly rounded on the luminal surface which thus appears scalloped.

Small, widely spaced mucosal folds jut into the lumen of the active gland. They rarely have secondary folds. They tend to be thick and round, in distinct contrast, for example, to the delicate narrow folds in the vesicular gland. A thin capsule of circular smooth muscle (tunica muscularis) surrounds each tubule and frequently abuts on the epithelium. The secretion of the active gland is granular and only moderately eosinophilic. Small, bright red droplets are dispersed throughout.

When the gland is inactive, its lumen is tiny, empty and lined by an epithelium comprised of (1) inactive cuboidal cells with large dense nuclei, and (2) larger cells that protrude into the underlying lamina propria. The latter occur in small groups, commonly adjacent to mucosal folds. They have considerable and poorly stained cytoplasm and central dense nuclei. The lamina propria is thick and highly cellular. The tunica muscularis consists of two layers of highly cellular dedifferentiated smooth muscle.

Dorsal Prostate Gland.--When active, this gland is a cluster of large acini, each surrounded by a thin capsule of smooth muscle (tunica muscularis).

Each acinus is lined by a simple cuboidal or low columnar epithelium. Nuclei in these cells are vesicular and basal. The apical cytoplasm is finely strippled and eosinophilic. This gland resembles the coagulating gland, but has fewer folds of the mucosa and smaller tubules. Some acini have no mucosal folds. The secretion is only faintly eosinophilic and frothy, but is sprinkled with bright red droplets.

The inactive gland is similar to the inactive ventral prostrate and ampullary glands and will be described with them below.

Ventral Prostrate Gland.--This compound tubular gland also consists of a cluster of tubules, each surrounded by a thin tunica muscularis. When active, each tubule is lined with a crowded simple cuboidal to columnar epithelium, the cells of which have basal vesicular nuclei and poorly stained, basophilic cytoplasmic granules in haematoxylin and eosin preparations. The height of the epithelium is inversely related to the volume of secretion in the lumen of the tubule. Each tubule is distended with an avidly eosinophilic (bright red) homogenous secretion similar to that of the vesicular gland. A layer of vesicles frequently separates the secretion from the epithelium. The eosinophilia, together with the small cluster of tubules that comprise the ventral prostate gland, are diagnostic. Few mucosal folds occur in the tubules and those present are extemely small and round.

Our description of the ventral prostate differs from that presented by Snell (1941) for the house mouse but instead resembles his description of the ampullary gland.

Ampullary Gland.--The numerous tubules of this compound gland are notably large and polygonal in section when the gland is active. The height of the epithelium varies considerably both among and within tubules, consisting of low cuboidal to crowded high columnar cells similar to those described above in the dorsal and anterior prostate glands. Nuclei are vesicular and basal. The apical cytoplasm is uniformly eosinophilic. Many tubules lack mucosal folds, but others have delicate folds of variable length. There is almost no lamina propria, and the thin circular layer of smooth muscle that constitutes the muscularis abuts on the epithelium of each tubule. Lumens, even in highly active glands, are often empty. The secretion, when present, consists of large masses of a highly vacuolated homogenous material, moderately stained with eosin and confined to the center of the lumen. It resembles that *illustrated* by Snell (1941) for the ampullary gland of the house mouse, but differs considerably from his *description* of the gland (*ibid*).

Inactive prostate and ampullary glands consist of tiny acini with minute lumens that are lined by a simple layer of cuboidal cells containing dense nuclei. Little and poorly stained cytoplasm is present. The epithelium is surrounded by a thick capsule of smooth muscle (tunica muscularis) which is generally highly cellular, circularly oriented, and dedifferentiated.

Seasonal Changes in the Accessory Sexual Glands of Male Deer Mice

Quantitative information concerning seasonal changes in the dimensions of the accessory reproductive glands of deer mice appears in Table 21.1.

Morton (in Lewis *et al.*, 1978) has shown that the annual reproductive period of male deer mice near Colstrip extends from mid-March through mid-September. Testicular and seminal vesicle weights are highest between March and August, suggesting maximal performance of the male reproductive system during this period. Kern in the same report (Lewis *et al.*, 1978) found active leydig cells in the testis between December and the following August and progressive increases in the diameter of tubules in the epididymis between March and June, followed by a brief decline in July and recovery in August. Seminiferous tubules in the testes were also enlarged between March and August. Development of the epididymis and seminiferous tubules depends on androgens produced by Leydig cells. The data suggest that the testes begin to secrete substantial and increasing amounts of androgen in February or March, and continue to produce enough to maintain the reproductive apparatus through August.

These findings concerning the steroidogenetic activity of the testis are consistent with the additional data presented here for the seminal vesicle, coagulating gland, and ventral prostate gland of the same mice. During 1974, the diameter of the vesicular gland was high in July and August, then diminished; diameters of the coagulating gland and ventral prostate were similarly large between July and September, then shrank abruptly. During 1975, the seminal vesicle was enlarged between April and August; the other glands between March and August (compare diameters during 1975 with those shown for October and December of 1974).

Fluctuations in the diameters of accessory glands during April, May, July, and/or August suggest that cyclic discharges of androgen from the testes occur during the breeding period. Changes in the head and tail of the epididymis of these mice (Lewis *et al.*, 1978) support this interpretation, although no peaks in size of the epididymis occur early in the breeding period.

Our specimens tended to have larger testes and seminal vesicles in May and June than in July and August (Lewis *et al* ., 1978). This trend is not present in the histometry of the seminal vesicle, coagulating gland, or ventral prostate (Table 21.1). The trend is observed, however, in the diameter of the seminiferous tubules of immature deer mice; diameter of tubules in the head of the epididymis of immature mice; and in the diameter of tubules in the tail of the epididymis of adults (see Tables 6.24-6.29, p. 248-253, of Lewis *et al* ., 1978).

Our data generally support Morton's suggestion (*ibid*) that immature males born early in the reproductive season breed later in the same season. The size of acini in the ventral prostate gland of immature males in June averages 360.0 µm (n = 2). This value is clearly within the size range of reproductively active adults (March-August interval in Table 21.1). Also, the diameters of the anterior prostate gland and seminal vesicle of immature males are within or just below adult size and sperm are frequently present in the seminiferous tubules and the epididymis of these males (Lewis *et al.*, 1978).

TABLE 21.1. SEASONAL CHANGES IN THE SIZE OF SELECTED ACCESSORY SEXUAL GLANDS OF ADULT MALE *Peromyscus maniculatus* COLLECTED AT COLSTRIP DURING 1974 AND 1975

Month - Year		Diameters of the Vesicular Gland*		Diameter of the Anterior Prostate Gland (μm)	Diameter of the Ventral Prostate Gland (μm)
		Average Diameter (μm)	Maximal Diameter (μm)		
Jul	1974	1968.0 (1)	2880.0 (1)	300.0 (2)	323.8 ± 40.1 (4)
Aug	1974	1656.5 ± 139.1 (5)	2265.6 ± 310.8 (5)	374.8 ± 39.1 (5)	252.8 ± 37.8 (4)
Sep	1974	1268.6 ± 106.1 (8)	1587.0 ± 125.1 (8)	300.7 ± 31.3 (7)	202.3 ± 27.0 (8)
Oct	1974	1398.0 ± 368.6 (5)	1761.6 ± 366.0 (5)	180.6 (3)	119.8 ± 47.1 (4)
Dec	1974	635.8 ± 75.1 (4)	756.0 ± 103.9 (4)	78.7 (2)	90.5 (2)
Mar	1975	1380.0 (2)	2196.0 (2)	366.5 (2)	272.7 (3)
Apr	1975	2300.6 ± 162.1 (7)	2904.0 ± 172.0 (7)	380.9 ± 47.4 (7)	254.3 ± 39.3 (7)
May	1975	1893.4 ± 150.2 (10)	2575.2 ± 210.4 (10)	396.0 ± 48.3 (8)	356.1 ± 31.4 (7)
Jun	1975	2034.0 ± 139.7 (18)	2596.0 ± 198.4 (18)	314.6 ± 26.8 (12)	287.3 ± 27.2 (10)
Jul	1975	2287.9 ± 153.1 (10)	2800.8 ± 202.0 (10)	336.6 ± 55.5 (9)	311.4 ± 18.5 (10)
Aug	1975	2183.3 ± 265.7 (7)	2842.3 ± 325.1 (7)	381.9 ± 85.3 (7)	280.1 ± 39.9 (7)

* Values in the table are Means ± SEM (n).

Ovary

Histology-Preliminary Introduction

Ova undergo limited growth and two meiotic cell divisions while in the ovary. More conspicuous concurrent changes take place within the follicles in which the ova mature. In the early developmental stages, the ova are near the surface of the ovary and are surrounded by one to few layers of flattened "follicle cells" which, together with the immature egg, are called primordial follicles. As the ovum grows and divides, the surrounding follicle cells proliferate and become many layers deep and a connective tissue capsule (theca) forms on the outer surface of the mass. Thus, a "primary follicle" is formed. Liquid-filled spaces now appear among the follicle cells, increase in size, and eventually coalesce to form a single large cavity (the antrum) within the follicle. Follicle cells now form a layer several cells in depth around the antrum. This layer is called the zona granulosa or membrane granulosa and the cells are now called granulosa cells. The theca is now thicker and consists of an inner vascular layer (theca internal) containing clusters of hormone-producing gland cells, and an outer layer (theca external) of connective tissue and smooth muscle. In contrast to the condition in most other rodents that have been studied, the theca of the deer mouse is usually thin and not well differentiated into layers. It usually consists of several to many layers of vascular connective tissue with few gland cells and little muscle.

We refer to follicles in which cavities have appeared and/or coalesced to form a small antrum as "antral follicles", and those with large antra into which the oocyte and an investing layer of granulosa cells project as "mature" or "Graafian follicles". There is considerable ambiguity in the literature concerning the names of follicles at various stages of development and definitions are thus particularly important. Pedersen and Peters (1968) have proposed a uniform system of nomenclature to resolve the problem. Wherever possible, we have included their designations for clarity.

After ovulation, the ruptured follicles collapse and more or less fill with blood from the ruptured thecal blood vessels and are then termed corpora hemorrhagica. The blood is rather quickly replaced by granulosa cells which increase in size and obliterate the original antrum, transforming the follicles into an endocrine gland, the true corpora luteum (TCL) which produces progesterone.

True corpus lutea are generally nonfunctional, transient structures in small rodents such as *Peromyscus* unless the female becomes pregnant, in which case they remain active for some time. Additional follicles in pregnant mice develop into accessory corpora lutea (ACL) by a process similar to that described for TCL except that the ova simply degenerates within the follicle. We find ACL to be uncommon in the ovaries of deer mice.

Eventually, TCL and ACL degenerate. Cells in the antrum diminish in size and number, undergo fatty degeneration, die, and are replaced by connective tissue. Ultimately, all that remains of them are small nonvascular clumps of scar tissue called corpora albicantes. Additional masses of cells that secrete progesterone occur in the ovarian stoma. They may be of thecal or granulosa origin (Harrison and Weir, 1977; personal observations). At any rate, they are called interstitial tissue and are especially prevalent during late gestation in many rodents.

Ovarian follicles may degenerate at any stage of development to form atresias which are viable endocrine glands that produce progesterone (Richards, 1978).

The endocrine component of the ovary is thus potentially large, consisting of estrogen-producing tissue (thecal gland cells and granulosa cells), androgen-producing tissue (thecal gland cells), and tissues that produce progestins (the granulosa layer of viable follicles; corpora lutea; artesias; interstitial tissue) (Bjersing, 1978; Richards, 1978).

Histology of the Deer Mouse Ovary

The following histological characteristics are diagnostic of the various stages of follicular development in the deer mouse ovary:

Primordial follicles of deer mice consist of an ovum and one to few enveloping layers of squamous (always!) follicle cells, average 24.67 ± 303 μm ($\bar{x}$ ± SEM; n = 18) in diameter, and characteristically occur in clusters near the ovary's surface.

Primary follicles consist of the ovum and one to several layers of surrounding follicle cells. If only one layer is present, the follicle cells are cuboidal or columnar (not squamous!). A theca of vascular connective tissue may be present. The egg itself is frequently surrounded by a well-defined, conspicuous zona pellucida. Mitotic figures commonly occur in the mass of follicle cells and in the theca. Primary follicles of deer mice are readily separated into small, intermediate, and large categories (Table 21.2).

TABLE 21.2. DIAMETER OF PRIMARY FOLLICLES OF DEER MICE

	Average Diameter (μm ± SEM) (n)	Range of Diameters (μm)
Primary follicles of small size	39.4 ± 3.6 (9)	<50
Primary follicles of intermediate size .	82.3 ± 3.2 (27)	50 - 105
Primary follicles of large size	150.8 ± 7.9 (18)	106 - 240

The primary follicles of deer mice correspond structurally to follicles of types 3-5 of Pedersen and Peters (1968).

Antral follicles of deer mice are generally larger than primary follicles. Many layers of follicle cells surround the egg. Among these are small fluid-filled spaces or a small, frequently slit-like antrum. If an antrum is present, the cumulus oophorus is broadly attached to the granulosa, *i.e.*, it barely projects into the antrum and most definitely not by a stalk. A conspicuous, but frequently thin theca is also present. "Early" antral follicles contain several small fluid-filled cavities, whereas "late" antral follicles contain no antrum. The diameters of the two types differ (see Table 21.3).

TABLE 21.3. DIAMETER OF ANTRAL FOLLICLES

	Average Diameter (µm ± SEM) (n)	Range of Diameters (µm)
Early antral follicles	204.2 ± 14.2 (16)	112 - 304
Late antral follicles	262.8 ± 8.8 (29)	167 - 352

The antral follicles of *Peromyscus* correspond structurally to follicles of types 6 and 7 of Pedersen and Peters (1968).

Mature (Graafian) follicles of deer mice are generally so large that they bulge from the ovary's surface. The antrum is large with a cumulus oophorus that projects from a stalk of granulosa cells. The membrana granulosa surrounding the antrum is many cells deep. The theca is broad and sometimes divisible into internal and external layers. We have divided these mature follicles into three size classes which presumably represent successive stages of growth (see Table 21.4).

TABLE 21.4. FOLLICLE SIZES REPRESENTING SUCCESSIVE STAGES OF GROWTH

	Average Diameter (µm ± SEM) (n)	Range of Diameters (µm)
Graffian follicles of small size	269.3 ± 5.8 (17)	204 - 296
Graafian follicles of intermediate size .	343.8 ± 5.9 (24)	310 - 391
Graafian follicles of large size	534.3 ± 20.0 (6)	462 - 592

The mature follicles of deer mice correspond structurally to follicles of type 8 of Pedersen and Peters (1968).

Corpora hemorrhagica of the deer mouse are generally ruptured and collapsed (or at least laterally compressed or irregular in shape) and filled with erythrocytes. Granulosa cells more or less crowd the erythrocytes into the center of the antrum and obliterate it.

Corpora lutea of deer mice form when the original antrum is filled with large cells, presumably of granulosa origin (Brambell, 1956). This "luteal" tissue is highly vascular, divided into small compartments by connective tissue, and is encapsulated by a stretched and thin theca in which there is little distinction between interna and externa.

We are able to classify the corpora lutea of deer mice into several functional categories based on cytological characteristics (see also Long and Evans, 1922; and Boling, 1942). Active corpora lutea (at peak secretory function) contain large round luteal cells with well-defined cell membranes; abundant, homogenous, deeply eosinophilic cytoplasm; and large vesicular nuclei with prominent nucleoli. Corpora lutea that have begun to regress (as defined by Brambell, 1956) contain vacuolated luteal cells. As regression progresses, the number and size of the vacuoles (lipid) increases, as does the number of cells with vacuoles. Nuclei are unchanged, but cell membranes become ill-defined. When completely regressed and nonfunctional, the corpora lutea contain small luteal cells that are vacant; with shrunken, dense and pycnotic nuclei; and ill-defined cell membranes. Neutrophils and macrophages commonly occur among the cells and there is an increase in connective tissue. They are also less vascular than active or regressing corpora lutea.

The corpora lutea of the deer mouse are readily classified into active, regressive, and nonfunctional types on the basis of size, as well as the cytological characteristics presented above (see Table 21.5).

TABLE 21.5. DIAMETER OF CORPORA LUTEA

	Average Diameter (µm ± SEM) (n)	Range of Diameters (µm)
Active corpora lutea	643.9 ± 15.6 (123)	296 - 1221
Regressing corpora lutea	(Table 21.6)	314 - 1036
Dysfunctional corpora lutea	337.0 ± 27.4 (14)	185 - 574

We have also been able to distinguish regressing corpora lutea at several stages of involution on the basis of their vacuolation and size (see Table 21.6).

TABLE 21.6. IDENTIFICATION OF REGRESSIVE CORPORA LUTEA BASED ON VACUOLATION AND SIZE

Degree of Regression	Vacuole Size	Number of Vacuoles per Cell	Number of Vacuolated Cells	Average Diameter (µm ± SEM) (n) (Range of Diameters, µ
		0	0	0
Very mild	Tiny	1 - 2	Few and Scattered	602.7 ± 21.4 (50) (329 to 1036
Mild	Small	1-few	Many and Scattered	626.7 ± 24.9 (40) (314 to 925)
Mild to moderate . . .	Small to Large	1-few	Most	687.9 ± 28.6 (11) (536 to 832)
Moderate	Small or Large	Several 1	Most or All	659.8 ± 22.7 (27) (444 to 925)
Moderate to heavy . . .	Variable	Many	Most or All	578.8 ± 28.5 (7) (500 to 722)

Two sets of corpora lutea were frequently found in a single ovary. We define those belonging to a single set as those at the same stage of development. Of the 89 adult ovaries that we examined, 13.5 percent contained two sets of corpora lutea. In each case, these appeared to be of successive sets. If one was fresh and active, the other consisted of regressing corpora lutea. On the other hand, if one set was regressing, the other was old and dysfunctional.

Corpora albicantes are spindle-shaped collagenous scars of variable size that frequently occur in large numbers in the ovaries of deer mice.

Atresias of all sizes occur in the ovary of the deer mouse. They exhibit numerous signs of degeneration such as shrunken oocytes with pycnotic nuclei, granulosa cells that are sloughing into the antrum, a collapsed antrum or one that contains connective tissue cells or inflammatory cells.

Interstitial tissue consists of eosinophilic cells free in the stroma of the deer mouse's ovary. The cells in these irregular well-vascularized masses have small dense nuclei, abundant lipoidal or poorly stained cytoplasm, and indistinct cellular membranes. They resemble the luteinized cells in dysfunctional corpora lutea.

Seasonal Changes in the Histology of the Ovary in the Deer Mouse

Seasonal changes in the ovary of adults during 1974 and 1975 appear in Table 21.7. Primary follicles were numerous in most ovaries throughout the year. Antral and Graafian follicles were also present in a high percentage of cases. Active corpora lutea were found in all months except the December-

TABLE 21.7. SEASONAL CHANGES IN THE OVARY OF ADULT *Peromyscus maniculatus*

Item in Ovary	Month and Year												
	Jul 74	Aug 74	Sep 74	Oct 74	Nov 74	Dec 74	Jan 75	Mar 75	Apr 75	May 75	Jun 75	Jul 75	Aug 75
n	4	7	15	10	8	2	3	2	7	6	8	8	9
Primary Follicles:													
% Females Possessing	100.0	100.0	100.0	100.0	87.5	100.0	100.0	100.0	100.0	100.0	87.5	87.5	100.0
Females (Number) with													
Small 1°F	1	2	8	6	5	2	1	1	1	2	2	5	4
Intermediate 1°F	4	5	15	8	5	1	3	2	7	6	7	7	7
Large 1°F	3	4	11	9	4	1	2	1	6	5	5	3	5
Females Examined	(4)	(5)	(15)	(10)	(7)	(2)	(3)	(2)	(7)	(6)	(7)	(7)	(9)
Antral Follicles:													
% Females Possessing	75.0	85.7	86.7	60.0	62.5	50.0	100.0	50.0	71.4	100.0	50.0	62.5	66.7
Females (Number) with													
Early A.F.	1	4	9	6	3	1	1	1	2	1	2	0	2
Late A.F.	2	2	8	3	1	0	3	1	5	5	4	4	5
Females Examined	(2)	(5)	(11)	(6)	(3)	(1)	(3)	(1)	(5)	(5)	(4)	(4)	(6)
Graafian Follicles:													
% Females Possessing	75.0	85.7	80.0	50.0	37.5	0.0	66.7	100.0	85.7	66.7	87.5	75.0	88.9
Females (Number) with													
Small G.F.	2	1	2	0	2	---	0	1	1	0	3	1	0
Intermediate G.F.	3	2	2	1	0	---	0	1	0	0	1	2	2
Large G.F.	0	3	1	2	0	---	2	1	4	1	7	5	6
Females Examined	(3)	(6)	(3)	(3)	(2)	---	(2)	(2)	(5)	(1)	(7)	(6)	(8)
Corpora Hemorrhagica:													
% Females Possessing	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	14.3	16.7	0.0	0.0	11.1
Corpora Lutea:													
% Females with Active C.L.	50.0	57.1	20.0	50.0	62.5	0.0	0.0	0.0	42.9	66.7	62.5	50.0	55.5
% Females with Regressing C.L.	50.0	28.6	60.0	20.0	0.0	50.0	0.0	100.0	57.1	50.0	50.0	50.0	44.4
% Females with Dysfunctional C.L.	0.0	0.0	20.0	10.0	12.5	0.0	0.0	0.0	0.0	33.3	0.0	12.5	0.0
Females (Number) with C.L. in													
Mild Regression	2	1	5	1	0	1	0	1	3	3	2	2	3
Moderate-Heavy Regression	0	1	4	1	0	0	0	1	1	0	3	2	1
Atresias:													
% Females Possessing	0.0	75.0	20.0	20.0	14.3	0.0	0.0	0.0	28.6	66.7	12.5	0.0	11.1
Interstitial Tissue:													
% Females Possessing	50.0	14.3	60.0	10.0	50.0	0.0	0.0	0.0	28.6	16.7	0.0	37.5	0.0
Supplemental Information:													
% Females Examined That Were Pregnant or Postpartum	75.0	71.4	28.6	57.1	0.0	0.0	0.0	100.0	55.6	66.7	77.7	40.0	40.0
% Females Examined That Had Uterine Scars	0.0	0.0	0.0	10.0	12.5	0.0	0.0	0.0	57.1	0.0	37.5	25.0	22.2

March interval (NB--we lack data for February). The distribution of corpora lutea suggests that the breeding season extended from March through December in 1974, but was suspended during winter. Late autumn breeding is probably unusual for populations of deer mice in southeastern Montana since it was not repeated in 1975, and in 1974 was accompanied by an unusually wet fall (Lewis *et al.*, 1978). However, deer mice breed year-round elsewhere (Brown, 1945; Asdell, 1964).

The ovary during January (including the absence of corpora lutea), is similar to that of voles during their nonbreeding (winter) season (Lecyk, 1962; Clarke and Forsyth, 1964; Clarke and Kennedy, 1967; Coutts and Rowlands, 1969), even though voles seem to be induced ovulators, and deer mice ovulate spontaneously (Asdell, 1964).

According to Brown and Conaway (1964), the corpora lutea of *Peromyscus* persist for about 2 months postpartum with little or no discernable change in structure. Morton (in Lewis *et al.*, 1978) found pregnant females among deer mice captured as late as October during 1974. If corpora lutea persist for at least 2 months before showing marked regression, then one would expect to find them in females as late as December 1974, which is the case (Table 21.7). The finding of two sets of corpora lutea in a substantial number of females in our samples also suggests that corpora lutea persist for some time. Even in unmated house mice, they may persist for two to four successive estrous cycles (Snell, 1941).

The amount of interstitial tissue in the murine ovary increases during late gestation (Brambell, 1956). Thus, interstitial tissue is abundant in reproductively active mice. It was also common in our specimens between April and September, but conspicuously absent through December and March (Table 21.7). Atresias were also absent in the latter months, yet abundant at times (June and August 1974) when interstitial tissue was not. In other words, the ovary of the deer mouse apparently contained progesterone-secreting structures only throughout the breeding season.

It would seem, then, that the atretic follicles and interstitial tissue of deer mice supplement the corpora lutea in the production of progesterone, which may be important in sustaining pregnancy after the corpora lutea begin to regress. We are surprised, however, that the incidence of atresia in any one deer mouse ovary is as low as it is (usually confined to one or at most a few follicles) given the fact that 75-77 percent of all ovarian follicles become atretic in the ovaries of rats and house mice (Arai, 1920; Mandl and Shelton, 1959; Jones and Krohn, 1961).

Additional results of the histological survey are the following:

1. Accessory corpora lutea rarely occur in the ovaries of deer mice. We found only one clear-cut example in the 89 adult ovaries that we examined.

2. The number of ovaries of immature deer mice available for study (11) was too small to permit us to generalize (Table 21.8). Notable, however, was the absence of atretic follicles and interstitial tissue in all 11. All contained primary follicles, frequently of large size and 10 also contained antral follicles. Six had Graafian follicles, some of which were of advanced age.

TABLE 21.8. RELATIONSHIP BETWEEN OVARIAN HISTOLOGY, OVARIAN WEIGHT, AND THE REPRODUCTIVE STATE OF ADULT AND IMMATURE *Peromyscus maniculatus**†

Reproductive State	N	1°F	A.F.	G.F.	At	CH	Corpora Lutea								Int. Tiss.	Ovary Weight As a Function of Body Weight (mg/g)‡
								Regressing								
							Active	Very Mild	Mild	Mild-Mod	Mod	Mod-Heavy	Heavy	Dys		
Immature	11	90.9	90.9	54.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.372 ± 0.048
Adult:																
Proestrus	0															
Estrus	4	100.0	75.0	100.0	50.0	0.0	50.0	25.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.425 ± 0.131
Metestrus	4	100.0	75.0	100.0	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.277 (3)
Diestrus	5	100.0	60.0	60.0	40.0	0.0	40.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0	40.0	0.337 ± 0.121 (4)
Anestrus	15	100.0	53.3	26.7	6.7	0.0	20.0	0.0	13.3	0.0	0.0	0.0	0.0	0.0	20.0	0.287 ± 0.034
Pregnant	26	96.2	65.4	84.6	26.9	3.8	46.2	15.4	26.9	3.8	15.4	3.8	0.0	7.7	19.2	0.313 ± 0.028
Immediately Postpartum	4	100.0	75.0	100.0	25.0	0.0	75.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.257 ± 0.117
Lactating Anestrus	11	90.9	81.8	81.8	9.1	0.0	27.3	0.0	18.2	18.2	36.4	0.0	9.1	36.4	54.5	0.228 ± 0.051

* All values except those for ovary weight are the percent of the females in each group that exhibit each item.

† Abbreviations in the table: AF = antral follicles; At = atresias; CH = corpora hemorrhagica; Dys = dysfunctional corpora lutea; GF = mature (Graafian) follicles; Int. Tiss. = interstitial tissue; Mod = moderate; 1°F = primary follicles.

‡ Ovary weights are presented as $\bar{X}$ ± SEM. Numbers in parentheses indicate sample size when it differs from that given on the left side of the table.

Changes in the Histology of the Ovary of Deer Mice During the Estrous Cycle

Data (1974-1975) in Table 21.8 are grouped according to reproductive state at time of capture (we will amplify later on the method we used to determine the mouse's reproductive state).

Primary follicles were well represented at all stages of the estrous cycle, and also in the ovaries of immature, pregnant, postpartum, and lactating mice. Atretic follicles were also common in the ovaries of reproductively active females. Curiously, active corpora lutea occur in many females at all stages of the estrous cycle. This suggests that corpora lutea do not immediately involute even when the female fails to become pregnant and is consistent with Snell's (1941) finding that the corpora lutea of house mice persist for two to four successive cycles.

We were quite surprised to find active corpora lutea in the ovaries of reproductively inactive (anestrus) mice, especially since few of these females had Graafian follicles. However, we expected to find corpora lutea at many different stages of regression in pregnant and lactating females. This is probably related to the fact that individuals were collected at various stages of gestation (which lasts 22-27 days) or lactation (which lasts up to 4 weeks), intervals in which the corpora lutea normally degenerate.

The average number of medium to large corpora lutea in the ovaries of pregnant deer mice (5.76 ± 0.01, $n = 34$) is slightly ($0.02 < P < 0.05$; t-test using square root transformations) higher than the number of embryos implanted in the uteri of the same mice. (5.09 ± 0.04), but is very similar to the overall mean litter size of deer mice collected at Colstrip during 1975 (5.75) (Lewis *et al.*, 1978). These findings suggest that fecundity and fertility are on the average the same, but that fecundity frequently exceeds fertility in individual mice. The latter is typical of the bank vole, *C. glareolus* , (Coutts and Rowlands, 1969) and has also been reported in two subspecies of *Peromyscus*, *P. m. gracilis* and *P. m. bairdii* (Asdell, 1964).

Uterine Horn and Vagina

Histology

The uterus and vagina of deer mice are structurally similar to those of other species of murine rodents as described by Parkes (1956). In both regions, the wall of the reproductive duct consists of three layers: endometrium, myometrium and perimetrium.

Changes in the Histology of the Vagina of Deer Mice During the Estrous Cycle

Diagnostic changes in the structure of the reproductive duct, particularly of its endometrium, occur during the 4 or 5 day estrous cycle of deer mice. These changes are similar to those exhibited by vaginas of rats and mice (Asdell, 1964). Vaginal smears are also similar (described for the house mouse by Long and Evans, 1922). This provides an accurate method of assessing the reproductive status of an individual deer mouse and is the rationale for including detailed studies of the vagina in the baseline data for *Peromyscus*. In addition, very early stages of pregnancy are easily missed during gross inspection of the uterus, as are unattached blastocysts in the uterine horns. Both are readily apparent in histological section making it possible to more closely define a female's reproductive state. This is the rationale for including the uterine horn in the baseline data.

The diagnostic characteristics of the vagina at each stage of the estrous cycle of the deer mouse are presented in Table 21.9. These are generally reliable for determining reproductive state. To these, we add descriptions of the histology of the vagina in pregnant and anestrous females. The description for pregnant animals is based on only three mice and is therefore tentative. In all cases, diagnostic changes occur primarily in the endometrium.

During pregnancy, the endometrium is similar structurally to that of a female in anestrous (Table 21.9) except that the epithelial cells may be mucified, as in house mice. The structure of the vagina of immature deer mice and of reproductively inactive adults is essentially the same. The endometrial epithelium is only one to three cell layers in depth (NB--this is the most reliable criterion for assigning individuals to the anestrous category). The most superficial layer consists of squamous or cuboidal cells with poorly stained cytoplasm, well defined cell membranes and large oval nuclei. The innermost layer blends into the highly cellular lamina propria. The epithelium lacks mitotic figures and rarely contains leukocytes. The underlying and lightly vascularized lamina propria consists of dense, highly cellular connective tissue, and also lacks inflammatory cells. The tunica muscularis has the two characteristic layers, but is thin and undifferentiated. Its undifferentiated state is the most reliable characteristic for distinguishing between diestrous and anestrous adults.

During lactation anestrous, the histology of the vagina is similar to the [illegible], except that (1) the endometrial epithelium consists of three to five cell layers and contains some leukocytes (neutrophils and lymphocytes), and (2) the tunica muscularis is thick and well developed.

We cannot emphasize enough the necessity of routinely including sections of the vagina in all future studies of *Peromyscus* in Montana (Table 21.10). Vaginal characteristics appear to provide the most reliable information concerning reproductive state.

Changes in the Histology of the Uterine Horn of Deer Mice During the Estrous Cycle

The histological structure of the uterine horn is not a reliable indicator of reproductive condition in deer mice. The relevant data are presented in Tables 21.9 and 21.11. One stage grades almost imperceptibly into the next. As in the vagina, most of the changes in the wall occur in the endometrial epithelium.

Because most of the slide material at our disposal consisted of uterine horns and so few vaginas were included (Table 21.11), we had to make inferences about stages of the estrous cycle for many of our animals from field

TABLE 21.9. HISTOLOGICAL CHARACTERISTICS OF THE UTERINE HORN OF *Peromyscus maniculatus*

Reproductive State	Endometrial Epithelium	Uterine Glands	Lamina Propria	Tunica Muscularis	Lumen
Immature	Simple crowded cuboidal or columnar layer; nucleus fills most of cell Few mitotic figures Few Leucocytes in lining (only lymphocytes)	Cuboidal to low columnar lining Tend to be small in diameter and inactive in appearance Lumens usually small and contain traces of secretion No mitotic figures in lining	Dense, cellular connective tissue No mitotic figures No leucocytes in stroma Lightly vascularized	Densely cellular Undifferentiated, except for layer of numerous and large blood vessels between muscle layers	Tiny
Adult: Proestrus[1]	Pseudostratified layer Traces of vacuolar degeneration Moderate numbers of mitotic figures No leucocytes in lining	Lined by truncated columnar cells Lumens small and empty Occasional mitotic figures in lining	Dense, cellular connective tissue Occasional mitotic figures No leucocytes in stroma Lightly vascularized	Densely cellular Just beginning to differentiate	Tiny
Estrus	High pseudostratified layer Cell membranes well defined Moderate numbers of mitotic figures Variable, but small numbers of leucocytes in lining	Lined by high columnar cells Lumens of variable size and contain traces of secretion Individual glands highly coiled (tortuous) Mild-to-moderate numbers of mitotic figures in lining	Highly cellular connective Occasional mitotic figures Variable infiltration of stroma with leucocytes (neutrophils and lymphocytes) Highly vascularized	Well-developed Highly vascular Hyperemic	Small and slitlike
Metestrus	High columnar to pseudostratified cells, frequently of unequal height, their rounded borders projecting unevenly into lumen Pillar cells (= discharged goblet cells) prominent and numerous Prominent vacuolar degeneration: vacant cells to cells with a supranuclear vacuole and above that a heavily stained eosinophilic layer facing the lumen Occasional mitotic figures	Lined by truncated columnar cells, some showing vacuolar degeneration Lumens of variable size with only traces of secretion No mitotic figures in lining Neutrophils and pillar cells in lining	Dense, cellular connective tissue No mitotic figures Variable, but large infiltration of neutrophils in stroma Highly vascularized by numerous hyperemic capillaries	Well developed Highly vascular Hyperemic Variable, but large infiltration of neutrophils in and between muscle layers	Variable: small to large in size

(continued)

TABLE 21.9. (continued)

Reproductive State	Endometrial Epithelium	Uterine Glands	Lamina Propria	Tunica Muscularis	Lumen
Metestrus	Conspicuous and numerous leucocytes in lining (neutrophils and lymphocytes)				
Diestrus	Variable: Low crowded columnar lining with cells of equal height in which most of the cell is filled with the oval nucleus or Epithelium of irregular height with rounded peaks and wide or narrow valleys, made up of many cells in which cell membranes are indistinct; nuclei are crowded and basal in the lining; superficial region of epithelium is eosinophilic cytoplasm No mitotic figures Occasional lymphycytes in lining	Lined by cuboidal or columnar cells Diameter of glands frequently small Commonly lack a lumen or have minute lumen	Dense, cellular connective tissue Small numbers of leucocytes infiltrate the stroma High vascularity; numerous hyperemic capillaries	Generall well developed, but beginning to differentiate in some cases Little leucocytosis	Small and slitlike
Anestrus	Simple crowded cuboidal or low columnar layer Nuclei fill most of epithelial cells; little cytoplasm No mitotic figures Occasional lymphocytes in lining	Lined by inactive low columnar cells; sometimes almost cuboidal Diameter characteristically small Lumens tend to be small and to contain traces of secretion	Dense, cellular connective tissue No infiltration of leucocytes Lightly vascularized	As in immature mice	Tiny
Pregnant (areas other than in the sites of implantation)	High columnar to pseudostratified layer Epithelial cells sometimes of equal height, but sometimes groups of cells with basal nuclei and eosinophilic apical cytoplasm collectively drawn out into sharp high peaks that protrude into the lumen Vacuolar degeneration common Occasional mitotic figures Occasional lymphocytes in lining	Lined by secretorily active columnar cells Lumens of variable, but frequently large size; empty or contain traces of secretion No mitotic figures in lining Vacuolar degeneration in lining	Dense, cellular connective tissue Variable leucocytosis	As in metestrus	

(continued)

TABLE 21.9. (continued)

Reproductive State	Endometrial Epithelium	Uterine Glands	Lamina Propria	Tunica Muscularis	Lumen
Immediately Postpartum	High columnar to pseudostratified lining Vacuolar degeneration common No mitotic figures Large numbers of leucocytes in lining (neutrophils and lymphocytes)	Lining of high truncated columnar cells Lumens empty Lining exhibits vacuolar degeneration and contains variable numbers of neutrophils	Dense, cellular connective tissue Marked leucocytosis (neutrophils and lymphocytes) Extravasation, in some places extreme Very highly vascular with numerous small capillaries and extreme hyperemia	As in metestrus	Large and distended
Lactation Anestrus	Simple crowded columnar lining with cells of unequal height extending unevenly into the lumen Nucleus occupies most of each cell; between it and lumen is a conspicuous, but small vacuole No mitotic figures Occasional lymphocytes in lining	Lined by active truncated, high columnar cells Lumens of variable size and contain traces of secretion No mitotic figures in lining	Dense, very cellular connective tissue Variable, but small infiltration of leucocytes in the stroma (neutrophils and lymphocytes) Highly vascular	Well developed Highly vascular Variable leucocytosis in and between muscle layers	Small or moderate in size

* This description is based on the uterine horns of two immature mice that were entering proestrus for the first time.

TABLE 21.10. HISTOLOGICAL CHARACTERISTICS OF THE VAGINA OF *Peromyscus maniculatus* AT VARIOUS STAGES OF THE ESTROUS CYCLE

State of the Estrous Cycle	Diagnostic Histological Features of the Vagina
Proestrus	Epithelium consists of a cornified stratified squamous layer under the surface and a superficial layer of mucified cuboidal or columnar cells with pycnotic nuclei Epithelium thick: 14-18 cell layers. Many mitotic figures in the epithelium. Few to no leucocytes in the epithelium
Estrus	Epithelium is a cornified stratified squamous layer Epithelium thick: 20-25 cell layers. Few mitotic figures in the epithelium. No leucocytes in the epithelium
Metestrus	Initially epithelium is a stratified layer with a sloughing cornified surface; the cornified layers disappear during late metestrus Epithelium thin: 9-12 cell layers. No mitotic figures in the epithelium. Leucocytes appear and gradually increase in number in the lamina propria under the epithelium; later, they become numerous in the epithelium
Diestrus	Epithelium is stratified, but not cornified Epithelium thin: 5-6 cell layers. No mitotic figures in the epithelium. Mild numbers of leucocytes in the epithelium

TABLE 21.11. RELATIONSHIP BETWEEN THE HISTOLOGY OF THE UTERINE HORN AND REPRODUCTIVE STATE OF FEMALE *Peromyscus maniculatus* *†

		Endometrial Epithelium ‡							Endometrial Glands			
Reproductive State	N	Type of Epithelium	Height (µm)	Number of Mitotic Figures	Presence of Inflammatory Cells	Type of Inflammatory Cells	Appearance of Basement Membrane	Vacuolar Degeneration	Number	Diameter (µm)	Type of Epithelium	Mitotic Figures in Epithelium
Immature	26	Cu-Co (12) Co (12) Co-Ps (2)	10.02± 0.53	0.40±0.20	0.08 ± 0.06	None (24) L (2)	D (2) D-F (1) F (18)	+ (5) ± (1) - (20)	2.17 ± 0.23	22.23 ± 0.89	Cu-Co (2) Co (22)	+ (2) - (24)
Adult:												
Proestrus	0	...	...	...	...	...	...	...	...	...	...	...
Estrus	4-5	Co-Ps (2) Ps (3)	23.66± 2.51	3.10±0.71	1.20 ± 0.73	None (2) L (2) PMN (1)	D (1) D-F (1) F (2)	+ (2) - (2)	4.40 ± 0.24	42.20 ± 4.82	Co (2) HiCo (2)	+ (4)
Metestrus	5	Co (1) Co-Ps (4)	22.70± 2.34	1.20±0.58	3.10 ± 0.78	None (0) L (4) PMN (3)	D-F (3) F (2)	+ (5)	4.00 ± 0.55	48.48 ± 5.17	Co (1) HiCo (4)	+ (2) - (3)
Diestrus	12	Cu-Co (2) Co (6) Co-Ps (3) Ps (1)	18.50± 1.47	0.33±0.26	1.38 ± 0.26	None (1) L (11)	D (2) D-F (5) F (5)	+ (5) ± (3) - (4)	3.83 ± 0.34	34.94 ± 2.46	Cu-Co (1) Co (4) HiCo (4)	- (12)
Anestrus	35	Cu-Co (11) Co (23) Co-Ps (1)	10.77± 0.45	0.00	0.39 ± 0.08	None (18) L (17)	D (6) D-F (7) F (22)	+ (3) ± (7) - (25)	2.93 ± 0.20	21.53 ± 0.76	Cu-Co (4) Co (31)	- (35)
Pregnant	25	Cu-Co (2) Cu-Ps (1) Co (7) Co-Ps (10) Ps (5) Pillar cells(11)	18.82± 1.13	1.02±0.28	1.64 ± 0.30	None (6) L (18) PMN (10)	D (7) D-F (10) F (7)	+ (14) ± (7) - (3)	3.22 ± 0.26	35.32 ± 2.20	Co (14) Co-HiCo(1) HiCo (9)	+ (3) - (21)
Immediately Postpartum	4	Co (1) Co-Ps (2) Ps (1)	29.75± 3.91	0.75±0.48	4.00 ± 0.71	None (0) L (4) PMN (4)	D (1) F (3)	+ (3) - (1)	2.75 ± 0.75	46.55 ± 4.83	Co (1) HiCo (3)	- (4)
Lactation Anestrus	14	Cu-Co (4) Cu-Ps (1) Co (5) Co-Ps (2) Ps (2)	18.11± 1.26	0.25±0.21	1.57 ± 0.16	None (0) L (14) PMN (3)	D-F (5) F (8)	+ (2) ± (3) - (8)	4.25 ± 0.24	38.38 ± 1.58	Co (5) HiCo (8)	+ (1) - (12)

* Abbreviations in the table: AcS = acellular secretion; An = anestrus; C = cells; Co = columnar; Cu = cuboida; D = distinct; Drb = debris; D-F = distinct in some places, but faded elsewhere; Df = differentiated; Di = diestrus; EC = epithelial cells; Es = estrus; F = faded; I = lumens of intermediate size; L = lymphocytes; L-D = lumens that are large and distended; Met = metestrus; Muc = mucified; PMN = neutrophils; Pro = proestrus; Ps = pseudostratified; RBC = red blood cells or erythrocytes; S-Sl = lumens that are small and slitlike; St = stratified; T = lumens that are tiny; Tr = just beginning to differentiate; UDf = undifferentiated or dedifferentiated; (+) = present and obvious; (±) = rare; (-) = absent.

† Values in parentheses in the table indicate the number of mice exhibiting the characteristic (i.e., sample size); other values are Means ± SEM.

‡ Mitotic activity of the endometrial epithelium, presence of inflammatory cells in the endometrial epithelium, the number of endometrial glands, the amount of secretion in the endometrial glands, the vascularity of the lamina propria, the degree of infiltration of the lamina propria with inflammatory cells, and the amount of material or fluid in the lumen of the uterine horn were each rated on a scale of 0 (non) to 5 (very marked or very numerous).

(continued)

TABLE 21.11. (continued)

Reproductive State	Endometrial Glands* (continued)	Lamina Propria of Endometrium*				Myometrium*		Lumen*			Uterine Weight as a Function of Body Weight (mg/g)	Repro-ductive State of Vagina
	Amount of Secretion Present	Vascularity	Infiltration of Inflamma-tory Cells	Type of Inflamma-Cells	Width (µm)	Width (µm)	Degree of Differen-tiation	Size	Contents (Amount)	Nature of Contents		
Immature	0.48±0.15	1.96 ± 0.21	0.12 ± 0.08	None (24) L (2)	91.29 ± 5.63	62.31 ± 2.82	UDf (25) Tr (1)	T	0.19 ± 0.08	None (21) AcS (2) Deb (4) C (1)	1.366 ± 0.398	Pro (1) Di (1) An (13)
Adult: Proestrus												
Estrus	2.00±0.77	2.80 ± 0.49	1.60 ± 0.68	None (2) L (1) PMN (3)	278.06 ±34.20	134.22 ±15.70	Df (4)	S-Sl	0.40 ± 0.28	None (3) AcS (2)	2.651 ± 0.352	Es (1)
Metestrus	0.40±0.24	3.20 ± 0.58	2.40 ± 0.24	None (0) L (2) PMN (5)	215.00 ±48.76	162.20 ±15.83	Df (5)	S-Sl (3) I (1) L-D (1)	0.40 ± 0.40	None (4) EC (1)	4.317 ± 0.286	Met (2)
Diestrus	1.75±0.33	2.67 ± 0.33	1.08 ± 0.34	None (6) L (6)	202.09 ±29.73	141.24 ±10.34	UDf (5) Tr (2) Df (5)	S-Sl (11) I (1)	0.08 ± 0.08	None (11) AcS (1)	2.722 ± 0.340	Di (8)
Anestrus	1.09±0.15	2.27 ± 0.18	0.06 ± 0.06	None (34) L (1)	106.33 ± 6.23	73.54 ± 3.65	UDf (34) Tr (1)	T (32) S-Sl (3)	0.34 ± 0.08	None (22) AcS (9) Deb (4) C (5) PMN (1) RBC (1)	1.173 ± 0.114	Di (1) An (17)
Pregnant	1.48±0.22	3.52 ± 0.10	2.02 ± 0.28	None (4) L (19) PMN (15) RBC (1)	205.88 ±26.50	225.15 ±14.52	Df (24)		2.00 ± 0.38(23)	None (5) AcS (18) Deb (2) C (4) PMN (10) RBC (7) L (2) Sperm (1) Pigment(1)	14.425 ± 3.548	Pro (1) Met (1) Di (2) An (1)
Immediately Postpartum	1.00±0.41	3.25 ± 0.25	4.00 ± 0.41	None (0) L (3) PMN (4)	145.62 ±40.64	285.50 ±59.37	Df (4)	L-D (4)	1.50 ± 0.29	None (0) AcS (3) C (2) PMN (3) RBC (3) L (1) Sperm (1)	16.030 ± 3.529	Pro (1) Met (1)
Lactation Anestrus	1.50±0.27	3.21 ± 0.21	1.96 ± 0.29	None (1) L (13) PMN (7)	236.75 ±25.95	205.24 ±17.60	UDf (1) Df (12)	S-Sl (6) I (6) L-D (1)	0.57 ± 0.14	None (6) AcS (8) Deb (1) C (1)	4.195 ± 0.536(13)	Di (2) An (2)

data on: (1) body and uterine weight; (2) the uterus--scarring, distension with fluid, implanted embryos; (3) the vagina--perforate or inperforate at capture; (4) condition of the mammary glands--conspicuous grossly or lactating. Guidelines used to establish reproductive state in questionable cases are these:

1. Small rodents such as *Peromyscus* with short estrous cycles and spontaneous ovulation do not have a luteal phase during a typical estrous cycle. Hence, the presence of well developed corpora lutea probably indicates pregnancy.

2. Reproductively immature mice do not ovulate. Hence, a mouse of unknown age whose ovary contains corpora lutea is an adult.

3. Deer mice have a postpartum estrus during which they ovulate. If fertilized, the ova develop into blastocysts, which may remain free in the lumen of the uterine horn for 15-20 days while the female is actively lactating (Asdell, 1964). Furthermore, females are in a state of anestrous while lactating. Hence, a deer mouse whose uterus contains free blastocysts is probably in a state of lactation anestrus.

4. Mammary glands are enlarged and conspicuous only during lactation. Hence, those females with conspicuous mammary glands are probably in a state of lactation anestrus.

5. The vagina of the deer mouse is perforate only at copulation and parturition. Thus, individuals of unknown reproductive state with perforate vaginas are either in estrus or immediately postpartum.

6. Mice weighing 14 or more grams were classified as adults, whether their ovaries contained corpora lutea or not (see also Lewis *et al.*, 1978). Those weighing less than 14 g, but having corpora lutea or uterine scars were also classified as adults, their small body weights notwithstanding.

7. Vaginal histology is a reliable indicator of the reproductive state of a deer mouse.

Using the above criteria, the deer mice in our sample sort out into the various reproductive classes described in Table 21.9 and 21.11. A few additional notes are in order:

1. Determination of the reproductive state of deer mice cannot be made on the basis of distinctness of the basement membrane of the endometrial epithelium, nor vacuolar degeneration in the epithelium, although these criteria have been used in studies of other murine rodents (*e.g.*, Parkes, 1956).

2. The uterine horn of the anestrous adult and the immature deer mouse are essentially the same and represent the basal, undifferentiated state of the tube.

3. The endometrial epithelium, and the glands derived from it, are especially proliferative (mitotically active) during estrus.

4. The acellular secretion in the lumen of the gravid and immediately postpartum uterus is similar to that in the uterine glands and is likely derived from them.

5. The state of the vagina in pregnant females varies considerably. In our limited sample of five vaginas, mudification of the vaginal epithelium was consistently present, although such is reportedly characteristic of pregnant house mice (Gorbman and Bern, 1962).

6. Changes in height of the uterine epithelium of small rodents during the estrous cycle are usually subtle, small, or inconsistent (Allen, 1922; Allen, 1931; Clauberg, 1931). However, (see Table 21.9), such changes are among the more reliable discriminating characteristics of the uterine horn of deer mice. The epithelium is low (10-11 µm) during anestrus and in reproductively immature deer mice; intermediate in height (18-19 µm) during anestrus pregnancy, and lactation anestrus; and high (23-30 µm) during estrus metestrus and immediately postpartum.

7. Uterine weight is a function of body weight and may also be useful in determining the reproductive state of female deer mice. This criterion allows clear separations of the following groups, which are listed in order of increasing uterine weights: (immature and anestrus adults) < (estrous and diestrous adults) < (adults in metestrus or lactation anestrus) < (pregnant and immediately postpartum adults).

8. The maternal and fetal placenta of the deer mouse is similar to the respective portions of the placenta of the house mouse (described by Rugh, 1968).

Adrenal Gland

Histology

The adrenal gland of the deer mouse is structurally similar to that of mammals generally (Bloom and Fawcett, 1975) and murine rodents specifically (Snell, 1941; Jones, 1957).

It has a core of medullary (chromaffin) tissue surrounded by a ring of cortical (interrenal) tissue. The medulla consists of cords of large polygonal cells with vesicular nuclei and abundant finely strippled cytoplasm. The cords are one or two cells across and separated from one another by large irregular venous channels, which are typically filled with erythrocytes. Even in preparations stained with haematoxylin and eosin, it is possible to distinguish two types of cells in the medullary cords. Most are a light burgundy color. However, other scattered cells are a deep purple color. The medulla is separated from the cortex by a thin capsule of connective tissue which is frequently difficult or impossible to identify in section.

The adrenal cortex exhibits typically mammalian zonation. Adjacent to the medulla is a zona reticularis (ZR) of loosley packed, polygonal cells, laced with small vascular channels (sinusoids). The irregular packing of cells in this zone is diagnostic. Cells tend to be small relative to those elsewhere in the cortex. They have vesicular or dense (sometimes pycnotic) nuclei. The cytoplasm contains moderately eosinophilic granules. Also found in this zone are scattered cells filled with a golden brown pigment (ceroid).

External to the ZR is a wider zona fasciculata (ZF) which is composed of narrow palisade-like columns of cells, radially arranged with respect to the medulla and separated from one another by fine vascular channels (sinusoids). Each column is one to three cells across (most commonly only one cell). The cells are generally large and square. Nuclei are quite irregular in size and vesicular. The cytoplasm is abundant and filled with eosinophilic granules giving the cells a diagnostic uniform red appearance.

The outermost and narrowest region of the cortex is the zona glomerulosa (ZG). It is not always clearly demarcated from the ZF. It consists of small clusters of cells surrounded by narrow vascular channels. The cells themselves are small and round with vesicular nuclei and little cytoplasm.

Sexually immature and nulliparous adult female mice are reported to have additional cortical zones, the most common of which is the X zone (Howard, 1927; Jones, 1957). These disappear in adult males and in females during pregnancy. Such zones are most often juxtamedullary. So few immature mice were available for study that we largely ignored the X zone. However, our limited data indicate that an X zone persists in reproductively active cycling female deer mice (n = 3) until they become pregnant. It is absent in pregnant (n = 6), postpartum (n = 1), and lactating mice (n = 1), as is a ZR. In its absence, the ZF is separated from the medulla by a broad hyperemic layer of loosely arranged connective tissue. Although no ZR is present, cells near the inner face of the ZF frequently have nuclei that are smaller than those in more peripheral areas of the cortex. In contrast, the ZR of reproductively active male deer mice is well-developed, but also frequently separated from the medulla by a hyperemic region of loosely organized connective tissue. (We had no immature male deer mice for comparison with adults.) Structurally, the X zone is similar to the ZR of adults.

Cortical cells frequently contain lipoidal vacuoles of variable size and number. Vacuoles also occur in medullary cells in some cases.

The entire gland is encapsulated by several closely applied, thin layers of connective tissue.

Seasonal Changes in the Adrenal Gland of Deer Mice

Seasonal changes in the histology of the adrenal gland are compiled in Table 21.12, from which we draw the following conclusions:

1. The adrenal gland rarely exhibits pathological changes in these populations of deer mice. In our sample of 72 mice, only three had clearly diseased adrenal glands. One exhibited extensive fatty degeneration of the medulla. Two others contained infiltrations of neutrophils in the ZR. (Small infiltrations of lymphocytes do occasionally occur in the ZR and medulla.)

TABLE 21.12. SEASONAL CHANGES IN THE HISTOLOGY OF THE ADRENAL GLAND OF *Peromyscus maniculatus*. VALUES IN THE TABLE ARE MEANS ± SEM

Age/Sex Group	Reproductive Status	Month Year	n	Total X-sec. of Adrenal Sec. (mm^2)	Percent of Total Cross-Sectional Area Occupied by ZG*	ZF-ZR(X)*	M*	Adrenal Weight as a Function of Body Weight (mg/g)
Adult Males	Reproductively Active	Jul 74	7	2.12 ± 0.19	7.7 ± 0.4	79.9 ± 3.2	12.4 ± 3.6	0.540 ± 0.093
		Aug 74	7	1.99 ± 0.17	9.4 ± 1.1	77.9 ± 3.9	12.7 ± 3.7	0.491 ± 0.044
		Sep 74	7	2.03 ± 0.12	8.5 ± 1.0	78.1 ± 4.8	13.4 ± 4.9	0.552 ± 0.055 (5)
		Oct 74	7	1.78 ± 0.12	9.8 ± 1.4	74.1 ± 2.4	16.1 ± 1.7	0.411 ± 0.038
		Nov 74	3	1.73	10.9	72.6	16.5	0.549
		Dec 74	4	1.72 ± 0.06	15.3 ± 3.4	65.6 ± 3.7	19.0 ± 1.4	0.602 ± 0.084
		Jan 75	1	2.10	8.6	88.6	2.9	0.643
		Mar 75	3	2.79	6.6	80.0	13.4	0.961
		Jun 75	2	1.44	16.4	69.4	14.2	1.000
Immature Females	Reproductively Inactive	---	1	1.56	10.3	77.6	12.2	0.677
Adult Females	Anestrus (inactive)	---	2	2.16	8.0	78.7	13.4	0.424 (1)
	Cycling	---	4	2.46 ± 0.16	8.8 ± 1.4	79.5 ± 4.3	11.7 ± 3.0	0.667 ± 0.064
	Pregnant	---	18	2.39 ± 0.10	10.7 ± 1.6	75.3 ± 2.2	14.0 ± 1.6	0.696 ± 0.078
	Lactating Anestrus	---	6	2.81 ± 0.18	9.9 ± 1.8	78.5 ± 1.9	11.6 ± 2.1	0.713 ± 0.075 (5)

* ZG = zona glomerulosa; GF = zona fasciculata; ZR = zona reticularis; M = medulla.

† The degree of vacuolation, degeneration, or infiltration of cortical tissue was quantified according to the following scale:

Symbol	Definition	Assigned Numerical Value
0	None	0.00
±	Traces or rare	1.00
+	Some, several, few, or mild	2.00
++	Moderate numbers or amount	3.00
+++	Many, numerous, or heavy	4.00
++++	Very many, very numerous, or very heavy	5.00

(continued)

TABLE 21.12. (continued)

Age/Sex Group	Cortical Vacuolation			Degree of Cortical Degeneration*,†		Degree of Lymphycyte Infiltration*,†
	Number of Mice with Cortical Vacuoles (%)	Degree of Cortical Vacuolation*,†				
		Large Vacuoles in Cortical Cells	Small Vacuoles in Cortical Cells	Pycnotic Nuclei	Degenerate Cells	
Adult Males	28.6	0.00	1.14 ± 0.74 ZF(2)	2.86 ± 0.59 ZF(2) ZR(4)	0.57 ± 0.57 ZR(1)	0.29 ± 0.29 ZR(1)
	42.9	0.29 ± 0.29 ZF (2)	1.43 ± 0.92 ZF(2)	0.00	0.00	0.00
	28.6	0.00	0.86 ± 0.59 ZF (2)	2.57 ± 0.92 ZR(4)	0.00	0.00
	28.6	0.00	0.86 ± 0.59 ZF(1) ZR(1)	1.14 ± 0.74 ZF(1) ZR(1)	0.00	0.43 ± 0.43 ZR(1)
	0.0	0.00	0.00	2.00 ZR(2)	0.00	0.00
	75.0	0.00	3.00± 1.00 ZF(3)	3.25 ± 1.11 ZR(3)	0.00	0.00
	100.00	0.00	4.00 ZF	0.00	0.00	0.00
	33.3	0.00	1.33 ± 1.33 ZF(1)	1.67 ZF(1)	1.67 ZF(1)	0.00
	50.0	0.00	2.00 ZF(1)	0.00	0.00	0.00
Immature Females	100.0	2.00 ZF	4.00 ZF	0.00	0.00	0.00
Adult Females	0.0	0.00	0.00	1.00 ZR(1)	0.00	0.00
	0.0	0.00	0.00	1.50 ± 0.96 ZF(1) ZR(1)	1.00 ± 1.00 ZR(1)	1.00 ± 1.00 ZR(1)
	61.1	0.72 ± 0.30 ZG(1) ZF(5)	2.11 ± 0.49 ZF(8) ZR(2)	0.22 ± 0.22 ZF(1)	0.00	0.33 ± 0.23 ZR(2)
	66.7	0.00	3.17 ± 1.01 ZF(4) ZR(1)	0.33 ± 0.33 ZR(1)	0.00	0.00

2. Cross-sectional areas of adrenals of adult males suggest that the medulla was more active in October-December 1974 than during other months.

3. Monthly differences in the cross-sectional area of the ZF-ZR of adult males suggest that cortical function is relatively uniform except in early winter (December), when it is reduced.

4. On the basis of cortical vacuolation, more than 25 percent of adult males appear to have been under mild to moderate stress during December 1974 and perhaps in the January-August 1975 interval. In view of this and the tabulated data concerning degeneration of cortical tissue, adult males appear to be under mild to severe stress for much of the year.

5. Pregnancy and lactation appear to have imposed moderate stress on more than half of the females in these reproductive states.

6. There is no correlation between the cross-sectional area of the ZF-ZR and the lipid measurements for the same regions of the cortex.

7. Changes in the weight of the adrenal gland as a function of the body weight correspond to changes reported for rats of similar reproductive stages. As reported by Andersen and Kennedy (1933), adrenal weight is lowest in anestrous females, low during pregnancy, and highest at parturition, estrus and during lactation. In rats, these changes are associated with similar changes in the size of the adrenal cortex, but in deer mice this correlation is not apparent in the percent of the adrenal section consisting of cortex. However, it is reflected in the actual areas of the ZF-ZR in the female population (Table 21.13).

TABLE 21.13. AREA OF THE ZONA FASCICULATA AND ZONA RETICULARIS (ZF-ZR) IN FEMALE DEER MICE

Anestrous adults	(n = 2)	1.70	mm^2
Cycling adults	(n = 4)	1.96 ± 0.20	
Pregnant adults	(n = 18)	1.81 ± 0.10	
Lactating adults	(n = 6)	2.21 ± 0.18	

(values are means ± SEM)

8. Androgens are generally considered to inhibit the release of ACTH from the anterior pituitary gland of mammals, whereas estrogens promote its release. Consequently, the adrenals of male mammals tend to be smaller than those of females of the same species. However, this trend is not clearly shown in our data for deer mice. If androgens inhibit adrenal cortical func-

tion and presumably size, then one might expect changes in the size of the adrenal cortex and either testes size or the development of androgen-dependent male accessory sexual glands to be inversely related. Comparison of the data in Lewis *et al.* (1978) for the testes and seminal vesicles of our animals with the data on the adrenal cortex (specifically the ZF-ZR, *i.e.*, ACTH-dependent regions) tabulated in this report illustrates that for months in which sample size is three to seven, the area of the ZF-ZR and the weight of testes or seminal vesicles are directly related. All three are elevated in March-September, then decline to annual lows during December.

Spleen

Histology

In general, the tissue structure of the deer mouse spleen conforms with that of mammals (Bloom and Fawcett, 1975) and murine rodents specifically (Snell, 1941; Blaine and Conaway, 1969).

The organ is encapsulated by several layers of connective tissue and smooth muscle, from which muscular trabeculae extend into the splenic pulp. In section, the spleen is long and flat and trabeculae tend to be most numerous in the thinner regions where they extend from one side of the gland to the other. The prominence of smooth muscle in the trabeculae, and the extension of the latter from one side of the gland to the other, suggest that they may function to expel blood from the splenic pulp, *e.g.*, suggest that the spleen of *Peromyscus* is a blood cell reservoir.

The splenic tissue consists of red and white pulp. The white pulp (diffuse lymphoid tissue and germinal centers) is not usually present immediately beneath the capsule, but is well represented in the organ's center. Here, there are many arteries, each surrounded by white pulp, arranged in tandem along the long axis of the spleen. This queue extends from one end of the spleen to the other. Additional finer arteries and arterioles surrounded by thin cuffs of white pulp are numerous and widely scattered throughout the gland.

It is sometimes difficult to distinguish diffuse lymphoid tissue from germinal centers in the white pulp because all of the lymphoid tissue around a major blood vessel resembles one gigantic germinal center. Accordingly, we adopted the following criteria to distinguish germinal centers: (1) they exhibit light and dark hemispheres; and (2) are encapsulated by a dense layer of reticular cells and small lymphocytes. Except for their occasionally large size, the germinal centers are histologically similar to those of other mammals (see Bloom and Fawcett, 1975: 446-561). They are occasionally packed with large clear spaces containing macrophages (with phagocytized hemosiderin), cellular debris, eosinophils, and neutrophils.

The red pulp consists of (1) vascular (splenic) sinuses lined by simple squamous or cuboidal cells, and (2) intervening splenic cords, islands of tissue containing many small lymphocytes, reticular cells, and macrophages. Hemosiderin, when present, is usually in macrophages within the cords, rather than in the vascular sinuses. The cords may also house nests of plasma cells and medium to large sized lymphocytes, and occasionally neutrophils. They always contain hemocytoblasts and megakaryocytes (occasionally even metamyelocytes and megakaryoblasts), *e.g.*, stem cells that produce blood cells and platelets, respectively. These stem cells commonly contain hemosiderin. Since we did not see platelets (perhaps because of the extreme cellular density of the spleen), it is possible that some of the cells identified as megakaryocytes, especially those with large amounts of hemosiderin, are multinucleated giant cells. However, Snell (1941) notes that megakaryocytes are conspicuous and characteristic of the spleen of house mice.

Seasonal Changes in the Histology of the Spleen of Deer Mice

The data tabulated in Tables 21.14 and 21.15 provide quantitative information about the red and white pulp, respectively, of the deer mouse at various times of year. From the tables, we make the following general observations:

1. Platelet formation occurred year-round, but was depressed in December. (Observe changes in the population of megakaryocytes in the red pulp.)

2. Erythropoiesis and/or storage of erythrocytes in the spleen was elevated in July-August 1974. (Note rations of red to white blood cells and the percent of the spleen cells in the red pulp represented by erythrocytes and normoblasts.)

3. The destruction of erythrocytes was elevated in May and June, and the August-October interval. (This conclusion is based on the hemosiderin content of the spleen.)

4. Lymphopoiesis was elevated during September-November. (Note the percent of the cellular population represented by medium and large sized lymphocytes.)

5. The number of foreign bodies trapped in the spleen and the antibody production of the organ was high in August-November and perhaps in June. (This is suggested by the numbers of macrophages, neutrophils, plasma cells, and medium to large lymphocytes in the red pulp, as well as the number and mitotic activity of the germinal centers in the white pulp.)

6. On a seasonal basis, splenic weight declines during November and December. Spleen weight also shows considerable individual variation in *Peromyscus* , ranging between 1.498 and 16.337 mg/g of body weight. Such large variation is not unexpected (see Skryja and Clark, 1970). In house mice, for example, splenomegaly is found in subordinate males, in distinct contrast to dominant individuals, and is accompanied systemically by anemia and histologically by marked reduction in the white pulp, and increased formation of erythrocytes and megakaryocytes in the red pulp (Blaine and Conaway, 1969). However, if the differences in spleen weight in deer mice are a function of social status, then we might expect to find the following in enlarged spleens: (1) low percentages of small lymphocytes; (2) high percentages of erthrocytes, megakaryocytes, hemocytoblasts, and normoblasts in the red pulp; (3) large

TABLE 21.14. SEASONAL CHANGES IN THE HISTOLOGY OF RED PULP IN THE SPLEEN OF *Peromyscus maniculatus*. AGE AND SEX CLASSES ARE COMBINED IN EACH MONTH. CELL POPULATIONS ARE EXPRESSED AS A PERCENT OF THE TOTAL CELLS PRESENT UNLESS OTHERWISE INDICATED. VALUES ARE MEANS ± SEM

Month-Year	n	Small Lymphocytes	Medium and Large-Sized Lymphocytes	Plasma Cells	Macrophages	Neutrophils and Eosinophils	Erythrocytes	Normoblasts	Hemocytoblasts	Myelocytes or Metamyelocytes	Megakaryocytes	Reticular Cells	Endothelial Cells	Smooth Muscle	Megakaryocytes*	Hemocytoblasts*	Hemosiderin Content*	Ratio of Erythrocytes to Leucocytes (R/W)**
Jul 1974	12	26.7 ±3.6	4.6 ±2.4	0.0	4.2 ±1.4	0.4 ±0.3	40.1 ±2.9	0.2 ±0.2	1.2 ±0.6	0.0	0.8 ±0.4	14.9 ±1.6	6.0 ±1.4	0.4 ±0.3	1.83 ±0.34	2.67 ±0.43	1.75 ±0.33	1.33 ±0.17
Aug 1974	19	26.4 ±3.5	6.0 ±1.0	0.1 ±0.1	5.1 ±0.9	0.5 ±0.3	35.0 ±2.4	0.0	0.5 ±0.2	0.0	0.4 ±0.2	16.6 ±2.1	7.5 ±1.1	1.8 ±0.4	1.63 ±0.22	2.42 ±0.26	2.26 ±0.27	1.13 ±0.12
Sep 1974	19	22.2 ±1.9	13.9 ±2.3	0.1 ±0.1	7.8 ±1.6	2.4 ±0.6	21.1 ±2.4	0.1 ±0.1	0.6 ±0.2	0.1 ±0.1	0.4 ±0.2	20.4 ±1.9	9.2 ±1.2	1.6 ±0.4	1.68 ±0.31	2.37 ±0.32	3.00 ±0.30	1.03 ±0.13
Oct 1974	13	20.7 ±3.3	9.4 ±2.0	1.1 ±0.4	9.7 ±1.6	3.0 ±1.0	20.3 ±2.7	0.0	0.8 ±0.4	0.0	0.2 ±0.2	22.7 ±2.3	11.0 ±1.6	1.1 ±0.5	1.23 ±0.23	2.23 ±0.38	2.27 ±0.34	1.35 ±0.16
Nov 1974	6	19.6 ±2.3	14.2 ±2.6	0.4 ±0.4	10.0 ±1.9	2.5 ±1.3	20.8 ±3.5	0.0	1.7 ±0.8	0.0	0.4 ±0.4	17.5 ±1.9	12.5 ±2.5	0.4 ±0.4	1.67 ±0.33	2.83 ±0.48	1.67 ±0.44	1.25 ±0.21
Dec 1974	4	18.8 ±5.8	3.1 ±1.6	0.0	8.8 ±4.3	1.2 ±0.7	26.2 ±2.2	0.0	0.9 ±0.6	0.0	0.0	30.0 ±7.9	10.0 ±1.0	1.2 ±1.2	0.50 ±0.29	1.00 ±0.41	1.25 ±0.63	1.38 ±0.24
Jan 1975	1	22.5	5.0	0.0	7.5	0.0	17.5	0.0	0.0	0.0	0.0	37.5	10.0	0.0	1.00	4.00	0.00	0.50
Mar 1975	6	35.0 ±5.7	4.6 ±1.5	0.0	9.6 ±1.9	0.4 ±0.4	19.6 ±2.6	0.0	1.7 ±0.5	0.0	0.0	21.7 ±3.5	6.2 ±1.9	1.2 ±0.6	2.33 ±0.80	4.17 ±0.40	1.25 ±0.25	1.00 ±0.18
May 1975	3	26.9	3.3	0.0	17.8	0.0	28.4	0.0	0.0	0.0	0.8	15.5	5.7	1.6	1.33	3.67	3.83	1.33
Jun 1975	7	26.9 ±4.3	2.5 ±1.1	0.0	21.1 ±2.7	1.8 ±0.9	21.4 ±3.4	0.0	1.4 ±0.7	0.0	0.0	19.3 ±2.1	5.0 ±2.2	0.7 ±0.5	1.86 ±0.70	2.67 ±0.71(6)	3.36 ±0.28	0.93 ±0.07
Jul 1975	1	35.0	7.5	0.0	7.5	0.0	12.5	2.5	2.5	0.0	0.0	17.5	15.0	0.0	1.00	4.00	0.00	0.50
Aug 1975	4	19.0 6.2	4.1 ±1.1	1.2 ±1.2	17.6 ±4.4	0.0	24.4 ±6.9	0.0	2.4 ±1.0	0.0	1.1 ±1.1	21.4 ±6.6	4.0 ±1.0	4.7 ±1.0	3.50 ±0.50	3.50 ±0.50	3.25 ±0.32	1.12 ±0.43

* Subjective ratings concerning the number of megakaryocytes and hemocytoblasts, and the amount of hemosiderin, in each spleen have been transformed into numerical equivalents of this summary, viz:

Rating Scale	Numerical Equivalent
0 = none	0
± = traces (rare)	1
+ = mild (some or few)	2
++ = moderate	3
+++ = heavy (many or numerous)	4
++++ = very heavy (very numerous)	5

** Subjective ratings of the ratio of erythrocytes to leucotytes in the red pulp of the spleen have been transformed into numerical equivalents for purposes of this summary, viz:

Rating Scale	Numerical Equivalent
R << W	0.0
R > W	0.5
R = W	1.0
R > W	1.5
R >> W	2.0

TABLE 21.15. SEASONAL CHANGES IN THE HISTOLOGY OF WHITE PULP IN THE SPLEEN OF *Peromyscus maniculatus*. AGE AND SEX CLASSES ARE COMBINED IN EACH MONTH

Month-Year	n	Germinal Centers in White Pulp			Spleen Weight as a Function of Body Weight in mg/g ($\bar{x}$ ± SEM)
		Total Number ($\bar{x}$ ± SEM)	Range	Number with Pronounced Mitotic Activity and/or Lymphoblasts	
Jul 1974	12	16.67 ± 1.91	3 - 27	0	4.545 ± 0.469
Aug 1974	20	13.00 ± 1.18	3 - 23	0	4.192 ± 0.475 (14)
Sep 1974	19	18.21 ± 2.75	4 - 51	0	4.966 ± 1.240 (11)
Oct 1974	13	16.31 ± 2.02	4 - 31	0	3.899 ± 0.993
Nov 1974	6	25.83 ± 4.75	14 - 44	2	3.376 ± 0.451
Dec 1974	4	13.75 ± 2.56	8 - 19	0	2.128 ± 0.271
Jan 1975	1	14.00		0	
Mar 1975	6	17.67 ± 2.70	12 - 26	1	4.521 ± 0.907
May 1975	3	12.33	4 - 17	0	4.224
Jun 1975	7	15.57 ± 2.26	7 - 24	0	6.860 ± 1.433 (6)
Jul 1975	1	14.00		0	4.323
Aug 1975	4	20.75 ± 2.46	17 - 28	2	5.411 ± 0.471

numbers of megakaryocytes and hemocytoblasts in the red pulp; (4) high red to white blood cell ratios; and (5) relatively few germinal centers in the white pulp. In only four of the 12 mice with splenomegaly did three or more of the above characteristics occur. It appears that social status and other as yet unidentified factors contribute to differences in the size and histology of this organ in deer mice. For example, four of the five females with enlarged spleens were pregnant.

Liver

Histology

The deer mouse liver is structurally similar to that of mammals (Bloom and Fawcett, 1975) and mice specifically (Snell, 1941). It consists of large "classical" lobules that are indistinctly separated from one another by connective tissue. Where three lobules meet, they form a space, the triad, containing branches of the hepatic artery, portal vein, bile ducts and lymphatic channels. Radially arranged vascular channels (sinusoids) carry blood toward the center of each lobule and anastomose there to form a central or hepatic vein. Sinusoids tend to be narrow and are sometimes difficult to locate. They are lined by a simple squamous endothelium and large, conspicuous Küpffer cells which project into the lumen. They are commonly filled with blood cells, which were frequently refractile, poorly stained and had considerable associated hemosiderin. This suggests that blood stood in the tissue for some time before fixation. Characteristics of the liver cells are consistent with this interpretation. Küpffer cells also commonly contained hemosiderin and occasionally black specks which we assume are particles of carbon.

Each lobule is composed of large, polygonal hepatic cells that are organized into cords or plates that radiate like the fins of a paddle wheel from the central vein to the edges of the lobule. Each hepatic plate is one cell across and separated from the next by a sinusoid. The liver cells or heptocytes in the plates have finely strippled cytoplasm and one or two nuclei. They are sometimes vacuolated (infiltrated with fat), have poorly stained cytoplasm (indicating hydropic degeneration and/or glycogen depletion) or pycnotic nuclei (indicating cellular degeneration) or contain a brown pigment.

Autolysis was frequently present in our liver samples. However, the observed pattern varied considerably from one liver to another. These variations may indicate shifts in blood flow through the liver as a function of processing at or near the mouse's death.

The liver was also commonly infiltrated by small focal aggregates of lymphocytes, usually near the triads.

Several livers exhibited pathological changes such as cirrhosis (n = 1), epithelioid replacement of hepatic tissue (n = 2), infiltration of the hepatic parenchyma by giant cells (n = 1), cocci with or without inflammatory responses (n = 3), and localized hepatic degeneration accompanied by inflammatory responses (n = 2).

Seasonal Changes in the Histology of the Liver of Deer Mice

Seasonal changes in the histology of the liver are summarized in Table 21.16, from which we draw the following inferences: 1. Fat storage in the liver was uncommon; 2. In contrast, lymphocyte infiltration, usually focal in nature and largely restricted to the triads, was common; 3. Glycogen depletion, widespread when present, occurred during July-September 1974; 4. Widespread autolysis was common, especially during December 1974 and June 1975; 5. Pathologies were most common during September 1974. In combination, items 3-5 suggest that the energy demands and/or sources of stress on the mice were unusually high during June-September and December. Results of the adrenal survey (changes in cortical lipid) are consistent with this interpretation: they suggest that the mice were under considerable stress during December 1974 and January-August 1975.

6. Küpffer cells contained the most hemosiderin during May and August 1975 (but not during August 1974). If this reflects erythrocyte destruction, then the latter was high during the same months, and erythropoiesis might be expected to rise shortly thereafter. The results of the spleen survey are consistent with this interpretation: they suggest that erythropoiesis was high in July and August and that erythrocyte destruction was high during May-June and August-October.

7. Blood flow through the liver was elevated during September, October, and December 1974, and March 1975, but reduced in May-August 1975. Whether this reflects real differences in liver function or simply variation in processing of the tissues (*e.g.*, the time of day when the mice were killed or the speed with which the liver was fixed) is unknown.

Kidney

Histology

The histological structure of the kidney of deer mice generally conforms to descriptions presented in standard references (Bloom and Fawcett, 1975).

Renal corpuscles are mammalian in type with a simple squamous Bowman's capsule and glomerular capillaries. The juxtaglomerular apparatus is a conspicuous feature of the vascular pole of many of them.

The proximal portion of the renal tubule consists of convoluted and straight regions. The latter is considered to be the descending thick limb of the loop of Henle (Bloom and Fawcett, 1975).

1. Convoluted region--this is a round, relatively large tube, lined with a simple high columnar epithelium that has a conspicuous brush border and radial striations in the basal region of the cells. Their cytoplasm is avidly eosinophilic. The nuclei are sharply defined, but variable in position in the cell. The lumen of the tubule is irregular, either large and oval or slit-like. Proximal convoluted tubules are especially numerous in the outer cortex of the deer mouse kidney.

TABLE 21.16. SEASONAL CHANGES IN THE HISTOLOGY OF THE LIVER OF *Peromyscus maniculatus* .* VALUES IN THE TABLE ARE MEANS ± SEM. SEX AND AGE GROUPS HAVE BEEN COMBINED

		Characteristics of Hepatocytes				Pathology	
Month-Year	n	Fatty Infiltration	Glycogen Depletion	Autolysis	Pigment Content of Küpffer Cells	Lymphycyte Infiltration	Hyperemia
Jul 1974	13	0.31 ± 0.13	1.62 ± 0.51	0.85 ± 0.52	0.46 ± 0.14	0.85 ± 0.22	1.69 ± 0.60
Aug 1974	20	0.25 ± 0.16	0.65 ± 0.31	0.60 ± 0.17	0.35 ± 0.17	0.60 ± 0.17	2.00 ± 0.46 (19)
Sep 1974	16	0.19 ± 0.19	1.62 ± 0.52	0.88 ± 0.33	0.00	0.40 ± 0.20	4.62 ± 0.20
Oct 1974	14	0.29 ± 0.16	0.00	0.71 ± 0.29	0.92 ± 0.51 (13)	0.79 ± 0.39	3.57 ± 0.39
Nov 1974	6	0.00	0.00	0.83 ± 0.48	0.50 ± 0.22	0.50 ± 0.22	2.33 ± 0.95
Dec 1974	4	0.00	0.00	3.50 ± 0.87	0.25 ± 0.25	0.00	5.00 ± 0.00
Jan 1975	1	0.00	0.00	1.00	0.00	0.00	0.00
Mar 1975	6	0.33 ± 0.21	0.00	1.33 ± 0.56	0.17 ± 0.17	0.17 ± 0.17	3.67 ± 0.67
May 1975	4	0.00	0.00	2.00 ± 0.47	3.50 ± 0.78	0.00	0.75 ± 0.75
Jun 1975	7	0.00	0.00	3.17 ± 0.91	1.29 ± 0.61	0.57 ± 0.57	0.14 ± 0.14
Jul 1975	1	0.00	0.00	1.00	0.00	0.00	0.00
Aug 1975	4	0.00	0.00	1.25 ± 0.63	2.75 ± 1.30	0.25 ± 0.25	0.75 ± 0.75
Grand Mean ± SEM (n = 12)		0.11 ± 0.04	0.32 ± 0.18	1.43 ± 0.28	0.85 ± 0.33	0.34 ± 0.09	2.04 ± 0.52

* Each histological characteristic in the table has been estimated on a scale of 0 to 5, according to which 0 = absent; 1 = mild; 3 = moderate; and 5 = heavy or pronounded. The values in the table are averages of values assigned to the animals in each monthly sample.

2. Straight region--This region is similar structurally to the convoluted region, but is somewhat smaller in diameter. The epithelium is also lower, consisting of simple cuboidal or low columnar cells which have the same high affinity for eosin, basal striations, and brush border. The lumen is round or oval. These tubules lie in clusters within the inner cortex and at the junction of the cortex with the medulla.

Loops of Henle are very narrow, round tubules with a thin, simple squamous lining. They occur in groups of 12 to 15 among thick limbs (proximal and distal) and collecting tubules. They can be recognized by their small diameter and the fact that they are accompanied by vasa rectae, capillaries filled with red blood cells. (This portion of the renal tubule is traditionally called the thin limb of the loop of Henle.)

The distal portion of the renal tubule, like its proximal counterpart, has a convoluted and a straight region, the latter being the ascending thick limb of the loop of Henle.

1. Convoluted region--This region of the tubule is round in section and somewhat smaller than its proximal counterpart. It occurs in the same general area, however. Groups of these tubules are frequently interspersed with groups of proximal convoluted tubules in the cortex. They are recognized by their simple cuboidal to low columnar epithelium, the cells of which are characteristically irregular in height and have poor affinity for eosin, granular cytoplasm, and lack of brush border. The lumen of this region of the renal tubule is slit-like and stellate-shaped in section.

2. Straight region--Histologically, this region of the tubule resembles the distal convoluted region, but is somewhat smaller in diameter. It is lined by a simple squamous or low cuboidal epithelium, consisting of poorly stained, granulated cells. In contrast to the convoluted region of the distal tubule, the lumen is round, generally empty, and has an epithelial lining of uniform height. Straight regions of the distal tubule occur in the outer medulla and bordering inner cortex.

Renal tubules are connected with a system of collecting tubules including collecting and papillary ducts.

1. Collecting ducts--These occur in the medulla and extend into the cortex as medullary rays to pick up the more peripheral renal tubules. They are round in section and relatively small with a simple cuboidal (in some places almost squamous) lining. The epithelium is characterized by its irregular height, and its cells by their lack of staining (vacant cytoplasm); they have well defined cellular membranes and contain a large, well defined nucleus.

2. Papillary ducts--These are large, round or oval tubules that are lined with a simple low cuboidal or columnar epithelium. Epithelial cells are similar to those lining the collecting ducts, but may also contain a few stained granules. The lumen is small and polygonal in shape. Papillary ducts are confined to the medulla, especially to the papilla.

Seasonal Changes in the Histology of the Kidney of the Deer Mouse

Seasonal changes in the histology of the kidney are compiled in Table 21.17. The data suggest that proteinuria commonly occurs in the kidney of deer mice; that concretions are rare; and, as expected, that hyperemia is a constant feature of the kidney.

We found many cases (n = 10) in which blood had pooled in the kidney for some time. Blood vessels were choked with poorly stained, refractile erythrocytes and hemosiderin. We suspect that these are fixation artifacts. Consistent with these observations is the fact that many of the kidneys were poorly fixed. Pycnosis, for example, was commonly widespread, particularly in the deeper regions of the kidney.

The kidney frequently (44.4 percent) exhibited pathological changes. The incidence of pathology exceeds 50 percent of the sample in September, October, and November, 1974 and in May, 1975. The most common pathology (28.4 percent of our sample) was focal lymphocyte infiltration. In only five cases did lymphocytes surround foreign bodies (bacilli). However, they were frequently accompanied by other types of inflammatory cells, such as macrophages, neutrophils, and in one case plasma cells. Lymphocytic infiltration was confined to the renal cortex and the distribution of lymphocytes there suggests that they arose by perivascular cuffing.

Epithelioid replacement of renal tubules occurred in three mice. In these animals, the renal tubules were solid cords of cells encased by lymphocytes and neutrophils. The kidneys of two other mice exhibited intertubular edema.

REFERENCES

Allen, E. 1922. The Oestrous Cycle in the Mouse. Amer. J. Anat., 30(2):297-348.

Allen, J. M. 1960. A Histochemical Study of 3(B)-Sterol Dehydrogenase in the Adrenal Cortex of the Developing Mouse. Anat. Rec., 137(1):57-63.

Allen, W. M. 1931. I. Cyclical Alterations of the Endometrium of the Rat during the Normal Cycle, Pseudopregnancy, and Pregnancy. II. Production of Deciduomata during Pregnancy. Anat. Rec., 48(1):65-103.

Andersen, D. H., and H. S. Kennedy. 1932. Studies on the Physiology of Reproduction. IV. Changes in the Adrenal Gland of the Female Rat Associated with the Oestrous Cycle. J. Physiol., 76(2):247-260.

Andersen, D. H., and H. S. Kennedy. 1933. Studies on the Physiology of Reproduction. V. The Adrenal Cortex in Pregnancy and Lactation. J. Physiol., 77(2):159-173.

Andrews, R. V. 1970. Effects of Climate and Social Pressure on the Adrenal Response of Lemmings, Voles, and Mice. Acta Endocrinol., 65(4):639-644.

TABLE 21.17. SEASONAL CHANGES IN SELECTED ASPECTS OF THE KIDNEY OF *Peromyscus maniculatus*.* SEX AND AGE CLASSES ARE COMBINED IN EACH MONTH

Month-Year	n	Number of Mice with Protein In*						Number of Mice with Concretions In*			Degree of Hyperemia In*,†				Mice in Sample with Pathological Kidneys
		PCT	PCT-I	DCT	DCT-I	LH	RC	PCT	PCT-I	LH	C	M	(C)(M)	Whole Kidney	
Jul 1974	13	0	5	0	1	0	0	2	2	1	2.56 ± 0.31 (9)	2.33 (3)	3.00 ± 0.58 (4)	2.68 ± 0.28	5
Aug 1974	19	7	1	1	0	0	1	0	0	0	3.15 ± 0.31 (10)	2.50 (2)	3.78 ± 0.22 (9)	3.45 ± 0.20	4
Sep 1974	15	8	0	8	0	2	0	1	0	0	2.46 ± 0.41 (13)	1.25 ± 0.72(4)	1.33 (3)	2.53 ± 0.37	10
Oct 1974	11	7	0	5	0	0	0	0	0	0	1.25 ± 0.53 (8)	0.00 (4)	0.86 ± 0.40 (7)	1.45 ± 0.39	6
Nov 1974	6	2	0	3	0	0	0	0	0	0	2.00 ± 0.89 (5)	0.00 (2)	0.67 (3)	2.00 ± 0.73	5
Dec 1974	3	1	0	2	0	0	0	0	0	0	4.00 (2)		4.00 (1)	4.00	0
Jan 1975	1	1	0	0	0	0	0	0	0	0	2.00			2.00	0
Mar 1975	6	2	0	3	0	0	0	0	0	0	2.00 ± 0.82 (4)	0.00 (1)	2.00 (3)	2.33 ± 0.61	1
May 1975	4	0	0	1	0	0	0	0	0	0	3.00 (3)	3.00 (1)	4.00 (1)	3.25 ± 0.48	3
Jun 1975	2	0	0	1	0	0	0	0	0	0	4.00 (1)		2.00 (1)	3.00	1
Jul 1975	1	1	0	1	0	0	0	0	0	0	4.00 (2)		2.00 (2)	3.00 ± 0.58	1

* C = cortex; (C)(M) = cortex and medulla combined; DCT = distal convoluted tubule; DCT-I = inner (straight) region of distal convoluted tubule; LH = loop of Henle; M = medulla; PCT = proximal convoluted tubule; PCT-I = inner (straight) region of proximal convoluted tubule; RC = renal corpuscle.

† Hyperemia was estimated on a scale of 0 (none) to 5 (very marked). Values are means ± SEM (n).

Anthony, A. 1953. Seasonal Reproductive Cycle in the Normal and Experimentally Treated Male Prairie Dog, *Cynomys ludovicianus*. J. Morph., 93(2):331-370.

Arai, H. 1920. On the Postnatal Development of the Ovary (Albino Rat), with Especial Reference to the Number of Ova. Amer. J. Anat., 27(4):405-462.

Arata, A. A. 1964. The Anatomy and Taxonomic Significance of the Male Accessory Reproductive Glands of Muroid Rodents. Bull. Florida State Mus., 9(1):1-42.

Asdell, S. A. 1964. Patterns of Mammalian Reproduction, 2nd Ed. Comstock Publ. Assoc., Div. Cornell Univ. Press, Ithaca New York. 670 pp.

Bjersing, L. 1978. Maturation, Morphology, and Endocrine Function of the Follicular Wall in Mammals. In: The Vertebrate Ovary, Comparative Biology and Evolution, R. E. Jones, ed. Plenum Press, New York. pp. 181-214.

Blaine, E. H., and C. H. Conaway. 1969. The Role of the Spleen and Adrenal in the Anemia of Subordinate Fighting Mice. Physiol. Zool., 42(3): 334-347.

Bloom, W., and D. W. Fawcett, eds. 1975. A Textbook of Histology, 10th Ed. W. B. Saunders Co., Philadelphia, Penn. 1033 p.

Boling, J. L. 1942. Growth and Regression of Corpora Lutea During the Normal Estrous Cycle of the Rat. Anat. Rec., 82(2):131-145.

Brambell, F. W. R. 1956. Ovarian Changes. In: Marshall's Physiology of Reproduction, 3rd Ed., Vol. I, Pt. 1, A. S. Parkes, ed. Little, Brown and Co., Boston, Mass. pp. 397-542.

Brown, H. L. 1945. Evidence of Winter Breeding of *Peromyscus*. Ecology. 26(3):308-309.

Brown, L. N., and C. H. Conaway. 1964. Persistence of Corpora Lutea at the End of the Breeding Season in *Peromyscus*. J. Mammal., 45:260-265.

Christian, J. J. 1955. Effect of Population Size on the Adrenal Glands and Reproductive Organs of Male Mice in Populations of Fixed Size. Amer. J. Physiol., 182:292-300.

Christian, J. J., and D. E. Davis. 1964. Endocrines, Behavior and Population. Science, 146:1550-1560.

Clarke, J. R., and I. A. Forsyth. 1964. Seasonal Changes in the Gonads and Accessory Reproductive Organs of the Vole (*Microtus agrestis*). Gen. Comp. Endocrinol., 4(3):233-242.

Clarke, J. R., and J. P. Kennedy. 1967. Effect of Light and Temperature upon Gonad Activity in the Vole (*Microtus agrestis*). Gen. Comp. Endocrinol., 8(3):474-488.

Clauberg, C. 1931. Genitalcyclus and Schwangerschaft bei der weissen Maus. (Anatomische Studien an Ovarium, Uterus and Scheide.) Dauer des Genitalcyclus. Arch. Gynak., 147:549-596.

Coutts, R. R., and I. W. Rowlands. 1969. The Reproductive Cycle of the Skomer Vole (*Clethrionomys glareolus skomerensis*). J. Zool. Lond., 158(Pt. 1):1-25.

Dawson, I. M. P., J. Pryse-Davies, and I. M. Snape. 1961. The Distribution of Six Enzyme Systems and of Lipid in the Human and Rat Adrenal Cortex before and after Administration of Steroid and ACTH, with Comments on the Distribution in Human Foetuses and in Some Natural Disease Conditions. J. Path. Bact., 81(1):181-195.

Delost, P., and H. Delost. 1954. Existence d'une zone X surrenalienne chez le campagnol roussâtre (*Clethrionomys glareolus S.*). C. r. Seanc. Soc. Biol., 148:1788-1793.

Ewel, K. C. 1972. Patterns of Change in the Reproductive Organs of the Male Pocket Gopher, *Geomys pinetis*. J. Reprod. Fert., 30(1):1-6.

Gorbman, A., and H. A. Bern. 1962. A Textbook of Comparative Endocrinology. John Wiley & Sons, Inc., New York. 468 pp.

Harrison, R. J., and B. J. Weir. 1977. Structure of the Mammalian Ovary. In: The Ovary, 2nd Ed., Vol. 1, General Aspects, S. Zuckerman and B. J. Weir, eds. Academic Press, Inc., New York. pp. 113-217.

Howard, E. 1927. A Transitory Zone in the Adrenal Cortex Which Shows Age and Sex Relationships. Amer. J. Anat., 40:251-293.

Hrabe´, V. 1970. Seasonal Changes in Microscopical Structure of the Vesicular Gland and the Prostate of *Glis glis* (Gliridae, Rodentia). Zool. Listy, 19(3):249-260.

Jones, E. C., and P. L. Krohn. 1961. The Relationship Between Age, Numbers of Oocytes and Fertility in Virgin and Multiparous Mice. J. Endocrinol., 21(4):469-495.

Jones, I. C., ed. 1957. The Adrenal Cortex, 1st Ed. Cambridge Univ. Press, Cambridge, England.

Lecyk, M. 1962. The Effects of the Length of Daylight on Reproduction in the Field Vole (*Microtus arvalis* Pall.). Zool. Poloniae, 12(2):189-221.

Lewis, R. A. and C. W. Lewis. 1979. Terrestrial Vertebrate Animals as Biological Monitors of Pollution. In: Monitoring Environmental Materials and Specimen Banking, N. P. Luepke, ed. M. Nijhoff, The Hague. pp. 369-391.

Lewis, R. A., M. L. Morton, M. D. Kern, J. D. Chilgren, and E. M. Preston. 1978. The Effects of Coal-Fired Power Plant Emissions on Vertebrate Animals in Southeastern Montana (A Report in Progress). In: The Bioenvironmental Impact of a Coal-Fired Power Plant, 3rd Interim Report, Colstrip, Montana, E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 217-283.

Long, J. A., and H. M. Evans. 1922. The Oestrous Cycle in the Rat and its Associated Phenomena. Mem. Univ. Calif., 6:1-148.

Mandl, A. M., and M. Shelton. 1959. A Quantitative Study of Oocytes in Young and Old Nulliparous Laboratory Rats. J. Endocrinol., 18:444-450.

Parkes, A. S., ed. 1956. Marshall's Physiology of Reproduction, 3rd Ed., Vol. I: Pt. 1. Little, Brown & Co., Boston, Mass. 688 pp.

Pedersen, T., and H. Peters. 1968. Proposal for a Classification of Oocytes and Follicles in the Mouse Ovary. J. Reprod. Fertil., 17(3):555-557.

Quay, W. B. 1960. The Endocrine Organs of the Collared Lemming (*Dicrostonyx*) Captivity under Diverse Temperature and Light Conditions. J. Morph., 107(1):25-46.

Richards, J. S. 1978. Hormonal Control of Follicular Growth of Maturation in Mammals. In: The Vertebrate Ovary, Comparative Biology and Evolution, R. E. Jones, ed. Plenum Press, New York. pp. 331-360.

Rugh, R. 1968. The Mouse, its Reproduction and Development. Burgess Publ. Co., Minneapolis, Minn.

Skryja, D. D., and T. W. Clark. 1970. Reproduction, Seasonal Changes in Body Weight, Fat Deposition, Spleen and Adrenal Gland Weight of the Golden-Mantled Ground Squirrel, *Spermophilus lateralis lateralis*, (Sciuridae) in the Laramie Mountains, Wyoming. Southwest. Nat., 15(2):201-208.

Snell, G. D., ed. 1941. Biology of the Laboratory Mouse. Blakiston Co., New York. 497 pp.

Tähkä, K. M. 1979. Age and Sex Differences in the Distribution of Lipids, Tetrazolium Reductases and Hydroxysteroid Dehydrogenases in the Gonads and Adrenals of the Bank Vole (*Clethrionomys glareolus*): Histochemical Study. Biol. Reprod., 21(1):125-133.

SECTION 22

SEASONAL CYCLES IN BODY COMPOSITION, ORGAN SYSTEM FUNCTION AND ENERGETICS OF THE WESTERN MEADOWLARK IN SOUTHEASTERN MONTANA: A REPORT OF PROGRESS

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ABSTRACT

Results of a portion of a baseline investigation of grassland birds in southeastern Montana are reported and evaluated in relation to future energy development.

Mensural and compositional characteristics of the whole body and of selected organs of the western meadowlark (*Sturnella neglecta*) are treated. The types of systems and functions represented are those that reflect the condition, vigor, nutritional state, metabolic state and resource relationships of the study population.

PREFACE

This paper is a synopsis of a small portion of our baseline investigation of grassland birds in southeastern Montana in relation to further energy development. A monograph is in preparation that will more fully treat the mensural and compositional characteristics of the whole body and of selected organs of the western meadowlark (*Sturnella neglecta*) and four other species of birds. The monograph includes baseline information on postnatal development, reproductive biology, histology, population dynamics and community structure.

Results reported herein together with those of the overall investigation will permit evaluation of the above aspects of avian organization as a function of age, sex, season, and physiologic state in relation to the physical and biotic environment. The present analysis treats a number of types of systems and functions that reflect the condition, vigor, nutritional state, resource relationships, metabolic state, and net energy balance of the sample population as a function of environmental information or gradients, including potential changes in air quality and other anthropogenic disturbances related to future energy development in southeastern Montana. The data are essentially baseline in nature.

We believe that future air pollution effects research in the northern Great Plains will rely heavily upon such results. The work in its entirety represents a baseline evaluation of annual cycle and life cycle phenomena and the mechanisms that regulate such functions in relation to potential vulnerability to air pollution induced stress. See Lewis *et al.* (1976), for a more complete statement of objectives.

INTRODUCTION

During the first 3 years of the study (1974-76) large numbers of avian specimens were collected in Rosebud and Powder River Counties. Previously we reported preliminary information obtained on these specimens (Lewis *et al.*, 1976, 1978). The available data on western meadowlarks have now been consolidated in the present report in order to characterize more fully the status of this species in the southeastern Montana environment at a baseline or prepollution condition.

Data on bodily composition of all vertebrate classes have powerful utility as measures of condition, metabolic and energetic stress, and life history strategies (Connell *et al.*, 1960; Helms, 1968; Helms and Smythe, 1969; McNeil, 1969; Myrcha and Pinowski, 1970; Telford, 1970; Adolph and Hegeness, 1971; Aleksiuk and Stewart, 1971; Perkins and Dahlberg, 1971; Krulin and Sealander, 1972; Morton *et al.*, 1974; Morton, 1975; Fehrenbacher and Fleharty, 1976).

Changes in body composition are also adaptive and highly predictable measures of age and phase of the annual cycle (Morton, 1975; 1976). Thus deviations from normal patterns that are deleterious, should be easily detected and should provide strong evidence for presence of environmental perturbation. Indeed, we have good evidence (Lewis *et al.*, unpublished data) that during certain phases of the annual cycle (*e.g.*, reproduction, growth, molt), that at least some of the species under investigation are living close to the limit of their resources or are under substantial environmental stress that may condition their responses to pollutants. Special attention will be given to these processes and their implications. We hope eventually to determine the extent of pollution-related effects on small birds in the study area and to distinguish, to the extent possible, between direct and indirect air pollution effects and the effects of other human activities that might otherwise tend to confound our results (*e.g.*, effects of coal-mining, water use, increased human population density, use of herbicides and pesticides, *etc.*).

Western meadowlarks and other grassland birds are potentially valuable monitors of environmental disturbance because they live in a relatively simple environment wherein the effects of abiotic factors should be only slightly buffered by the biotic community and thus easily discerned (Weins, 1973; 1974). This relationship has been amenable to development of useful models for energy flow in grassland populations (Wiens and Innis, 1974).

The annual cycle is the fundamental temporal unit in most long-lived animals. The essential processes of life such as winter maintenance, migration, reproduction, and molt occur within a highly specific schedule such that conflict between major energy-requiring events and environmental fluctuations and among the events themselves is minimized. The precise duration and timing of annually occurring functions are therefore essential and comprise key loci for selection pressures that shape evolutionary tendencies toward optimizing an individual's fitness (Mewaldt and King, 1977).

Some of the organs examined in this study, vary appreciably in mass during the season (spleen, liver, gonads) whereas others (heart, lungs, kidneys) do not. Both types could have considerable worth as pollution monitoring systems. For example, significant deviation from normal values for static organs could be indicative of pathology. Organs that cycle do so within highly specific temporal boundaries. Departure from the usual schedule might be a harbinger of environmental perturbation. In either case, precise diagnosis of pathology will depend upon corroboration through additional assessment of function. Such evaluations would logically involve biochemical and histochemical characters and microstructural parameters such as cellular size, organization, and number. Conducting evaluations of this type is usually expensive in time and money. Highly developed techniques and specialized equipment are also required. Collection of organ weights, however, is a relatively straightforward process that can be accomplished by any worker with simple equipment and basic biological skills. Changes in organ weights signal qualitative or quantitative changes in organ structures and function and these indicate bases for further analysis of tissues that we have banked.

MATERIALS AND METHODS

Specimens were collected with a shotgun, sealed in plastic bags with appropriate labels, and retained frozen until analysis. In many specimens internal organs were removed for weighing and/or fixation. In such cases, dissections were performed at the field vehicle immediately after shooting or the specimen was placed on ice and transported to our field laboratory at Fort Howes Ranger Station, Powder River County, for dissection. Collecting was done in Rosebud County along Rosebud, Cow and Greenleaf Creeks within 15 km of Colstrip. Some specimens were collected in Powder River County, usually within 20 km of Fort Howes.

In the laboratory we employed standard biometric methods and whole carcass and organ analysis to evaluate compositional and mensural changes as a function of age, sex, season, and physiologic state in relation to the physical and biotic environment, (for details, see Lewis and Morton, 1976; Lewis *et al.*, 1976, and below).

Frozen specimens were thawed and analyzed as follows:

1. Molt classifications were determined by detailed observation, the method being adapted from Morton *et al* ., (1969). The just-thawed carcass was then dissected and processed all or in part as follows:

2. Liver--wet weight, dry weight, fat content, fat-free dry weight; to tissue bank.
3. Left kidney--wet weight, dry weight; to tissue bank.
4. Adrenals--wet weight, return to carcass.
5. Thyroid--wet weight, return to carcass.
6. Gonads--wet weight of testes, ovaries and oviducts, return to carcass.
7. Spleen--wet weight, return to carcass.
8. Gizzard--wet weight, return to carcass.
9. Integument (plumage)--dry weight (does not include beak or leg scutes), return to carcass.
10. Carcass--dry weight (wet weight determined in field on date of collection), dried tissue ground and aliquots used to determine fat content; remainder to tissue bank.

Specific techniques used in making the measurements outlined above:

1. Wet weights of organs were made on a torsion balance as soon as they were dissected free and blotted.
2. Grinding of carcasses for production of homogeneous aliquots was done in a Model 4-E Quaker City Laboratory mill.
3. Fat content was determined by placing specimens in thimbles and extracting them in a soxhlet apparatus with 1,2-dichloro-ethane for 24 hours. Samples were then dried at 75°C in convection ovens and the fat-free dry weight recorded.
4. Total nitrogen was determined by the standard micro-Kjeldahl technique.
5. Caloric content was measured by combusting aliquots of dried specimens in a Parr adiabatic calorimeter.
6. Mensural techniques on plumage and appendages were adapted from Baldwin *et al.*, (1931).

RESULTS

The western meadowlark is monotypic and considered to be a sibling species of the eastern meadowlark, *S. magna* (Lanyon, 1962). It breeds from the

Great Lakes region westward from Mexico to southern Canada. Since the turn of the century, in association with settlement and deforestation by man, there has been a significant extension of its range to the northeast (Lanyon, 1956). This expansion is apparently still progressing (Rohwer, 1973).

Meadowlarks may range into mountain parks and foothills but are better known as inhabitants of prairies and grassy plains. They winter in parts of Mexico and in southern U.S. border states but have been recorded as residing the whole winter as far north as Miles City, Montana (Cameron, 1907). They may begin arriving in Montana on spring migration in late February or early March (Weydemeyer, 1973), but an 18-year average first arrival time for Custer County was 30 March (Gross, 1958). Fall migration usually begins in October but a few birds are known to have departed Montana and similar latitudes as late as mid-November.

The time of reproduction is, of course, the single most critical period in any organism's annual cycle. In the present study primary evaluation of this period was made by the use of weights of reproductive organs used to define the normal limits of gonadal development and the temporal limits of the breeding period.

Gonads

Our collection of meadowlarks began in early April when the first individuals arrived in the study area. Most of the birds collected throughout April were adult males, 126 of 145 or 87 percent. Testes of males collected in April were often one-third to one-half the size of those collected during May and June (Table 22.1); the size difference was highly significant ($P < 0.01$, t-test of means). The first males to arrive quickly established and began defending territories. They were conspicuous because of their frequent bouts of singing from elevated perches.

Females were not regularly present until May, at which time their ovaries averaged more than 500 mg, significantly larger ($P < 0.01$) than those taken in April (Table 22.2). In both sexes, considerable gonadal growth thus occurred in many individuals after reaching the study area.

Judging from gonadal and oviduct weights, and follicular diameters, adults of both sexes typically maintained reproductive function until mid or late July (Tables 22.1 and 22.2). Gonadal involution in July was rapid, spanning only a few weeks in the population that we sampled.

A few fledglings were present during the first half of June but they could not be found with regularity until the last half of the month. There was no detectable seasonal change in gonad weights of juveniles (Tables 22.1 and 22.3). Note that data on meadowlark juveniles are presented according to sex. Differences in body size between the sexes were eventually evident to us (see beyond), and the data were therefore segregated.

In most avian species, the right ovary and oviduct do not persist beyond early development, and ovarian and oviduct weights referred to here include only those of the left side. Oviduct size and functional status is primarily

TABLE 22.1. SEASONAL CHANGES IN PAIRED TESTES WEIGHTS (mg) IN WESTERN MEADOWLARKS COLLECTED NEAR COLSTRIP, MONTANA, 1974-1978

		Adults			Juveniles	
	N	Mean	S.D.	N	Mean	S.D.
April 1-15	51	282.7	139.2	--	--	--
16-30	33	479.5	189.7	--	--	--
May 1-15	33	539.5	152.3	--	--	--
16-31	39	722.6	146.8	--	--	--
June 1-15	35	677.7	176.6	2	4.8	--
16-30	43	673.6	183.3	12	3.6	1.3
July 1-15	25	572.3	220.0	8	3.9	1.3
16-31	18	298.0	257.5	14	6.7	2.5
Aug. 1-15	13	16.4	7.1	11	8.0	2.9
16-31	7	10.5	4.4	29	5.2	2.5
Sept. 1-15	8	8.4	1.4	34	4.3	1.9
16-30	9	8.2	3.2	23	3.6	1.7
Oct. 1-15	4	21.0	20.2	6	4.0	1.5

under the control of ovarian hormones although it does have independent vascular and nerve supplies (see reviews by Lofts and Morton, 1973; Sturkie and Mueller, 1976). The cycle seen in adult female meadowlarks (Table 22.2), therefore, reflects seasonal changes in secretion of ovarian hormones (estrogens and progesterone). The diminutive size and lack of change in oviducts of juveniles indicates that their ovaries were nonsecretory from June through September (Table 22.3).

Body Weights and Body Composition

Body weights afford the most convenient standard for comparisons of body size and energetics (Baldwin and Kendeigh, 1938). Weights of meadowlarks tended to decrease during the season and did not swing upward until August (Table 22.4). Body weights of juveniles increased through time as one would expect from normal growth. Both sexes of juveniles were approximately equivalent to their adult counterparts in body mass by September (Table 22.4).

TABLE 22.2. SEASONAL CHANGES IN WEIGHTS (mg) OF REPRODUCTIVE ORGANS AND DIAMETER (mm) OF PRE-OVULATORY FOLLICLES IN ADULT WESTERN MEADOWLARKS COLLECTED NEAR COLSTRIP, MONTANA, 1974-1978.

		Ovaries			Oviducts			Pre-ovulatory Follicles		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	1	81.10	--	1	357.90	--	--	--	--
	16-30	12	107.78	49.76	13	275.23	166.55	58	2.22	0.55
May	1-15	35	560.00	839.50	35	1864.61	1824.54	141	3.53	2.28
	16-31	33	537.79	900.41	32	1575.46	1697.50	140	3.45	2.53
June	1-15	22	414.65	520.63	21	1102.49	1412.55	63	3.68	2.99
	16-30	15	519.35	694.80	17	1319.94	1503.95	63	3.86	2.91
July	1-15	15	108.97	218.07	17	356.02	826.28	19	3.60	3.21
	16-31	13	42.05	16.61	12	128.14	79.05	6	1.08	0.48
Aug.	1-15	6	15.05	10.14	5	57.08	29.39	--	--	--
	16-31	9	13.45	4.86	8	40.36	21.57	--	--	--
Sept.	1-15	10	20.04	11.54	9	51.55	30.67	--	--	--
	16-30	5	21.36	6.34	3	22.41	13.76	--	--	--
Oct.	1-15	1	10.91	--	1	23.54	--	--	--	--

No large seasonal fluctuations in measured body components were apparent in adult (Table 22.5) or juvenile (Table 22.6) meadowlarks, but a few trends occurred. Mean body weight, for example, of adult males decreased during the last half of the season. This corresponded temporarily with postnuptial molt (see beyond) but the possible physiological significance of this relationship may be complex in that a similar trend did not occur in females.

TABLE 22.3. SEASONAL CHANGES IN WEIGHTS (mg) OF REPRODUCTIVE ORGANS IN FEMALE JUVENILE WESTERN MEADOWLARKS COLLECTED NEAR COLSTRIP, MONTANA, 1974-1978

		Ovaries			Oviducts		
		N	Mean	S.D.	N	Mean	S.D.
June	16-20	11	3.11	1.78	13	7.00	2.85
July	1-15	8	5.04	3.08	8	10.61	1.57
	16-31	7	5.71	2.61	7	10.75	1.63
Aug.	1-15	8	3.90	2.77	9	8.32	2.47
	16-31	16	3.53	1.85	11	9.71	6.41
Sept.	1-15	19	5.06	2.75	18	10.36	5.79
	16-30	15	8.06	6.70	15	9.95	4.28

The nitrogen content of meadowlark carcasses (Table 22.7) and pectoral muscle (Table 22.8) was remarkably constant.

Water content of adult males tended to be low in early April (Table 22.5). Comparison of the early and late April means indicates that they are marginally different ($0.10 > P > 0.05$). This could be a real effect because newly arrived migrants are sometimes noticeably dehydrated (Zimmerman, 1965). Rehydration is probably complete within the first day after arrival, however, and one must be able to determine exact arrival schedules of individuals to have confidence in the carcass composition data. Unfortunately we do not have precise information on arrival times of individuals.

Apparently collections ceased before adults began premigratory fattening, although the three males collected in October were fatter than birds collected earlier (Table 22.5). October samples of juveniles did not have significantly more fat than those of September ($P < 0.05$) when all data on juveniles are treated together. Note that sex was undetermined in 42 juvenile specimens but, because of its inherent value, data on their body composition is included in Table 22.6).

TABLE 22.4. SEASONAL CHANGES IN BODY WEIGHTS (g) IN WESTERN MEADOWLARKS NEAR COLSTRIP, MONTANA 1974-1978

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	76	118.67	8.57	1	99.40	--	--	--	--	--	--	--
	16-30	50	116.61	6.36	18	92.38	6.56	--	--	--	--	--	--
May	1-15	46	113.66	6.47	41	93.91	6.76	--	--	--	--	--	--
	16-31	46	114.70	4.96	42	92.65	8.99	--	--	--	--	--	--
June	1-15	42	112.93	5.78	27	89.75	6.67	2	69.67	--	--	--	--
	16-30	51	110.56	5.93	29	89.22	9.61	14	87.83	9.87	15	71.37	9.16
July	1-15	28	110.54	5.87	29	86.81	5.69	15	94.28	11.05	9	76.68	7.31
	16-31	29	110.89	6.21	25	85.74	5.96	33	98.89	7.42	32	79.42	4.53
Aug.	1-15	22	115.21	5.02	18	87.58	6.77	31	104.23	8.45	30	81.47	8.94
	16-31	12	118.53	5.06	18	91.53	5.29	46	107.33	14.66	32	83.34	5.51
Sept.	1-15	12	116.79	5.70	19	89.38	4.59	44	110.34	6.48	37	90.21	9.20
	16-30	12	118.88	3.98	9	94.48	3.61	33	115.21	8.47	23	90.80	7.58
Oct.	1-15	3	131.70	13.04	--	--	--	11	121.366	7.76	4	94.33	1.77

TABLE 22.5. SEASONAL CHANGES IN BODY COMPOSITION OF ADULT WESTERN MEADOWLARKS, 1974-1976

		Water (% body weight)			Lean (% body weight)			Fat (% body weight)		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
MALES										
April	1-15	48	64.91	6.31	46	29.44	3.17	46	6.49	2.23
	16-30	33	66.24	1.87	30	28.66	2.11	30	5.14	1.32
May	1-15	19	67.48	1.89	18	28.58	1.52	18	4.30	1.14
	16-31	27	67.19	2.52	25	27.96	2.81	24	5.22	1.38
June	1-15	16	67.55	1.96	16	28.09	1.99	16	4.42	1.14
	16-30	33	67.12	1.62	21	28.14	1.34	31	4.72	1.00
July	1-15	13	70.33	3.64	12	24.97	3.16	12	5.09	1.77
	16-31	20	69.15	3.13	20	26.17	2.98	20	4.70	0.80
Aug.	1-15	9	70.15	2.15	6	25.11	3.10	6	4.54	1.34
	16-31	3	71.67	2.15	3	24.05	2.04	3	4.28	0.75
Sept.	1-15	9	66.53	11.68	9	25.92	4.88	9	4.34	1.18
	16-30	13	66.19	3.47	12	27.54	3.18	12	6.16	1.70
Oct.	1-15	3	61.49	2.85	3	27.97	1.27	3	10.55	4.03
FEMALES										
April	16-30	13	63.90	2.18	13	28.26	1.12	13	8.08	2.08
May	1-15	28	64.52	6.69	24	27.58	1.28	24	6.78	2.30
	16-30	22	67.86	3.10	22	26.67	2.53	22	4.82	0.93
June	1-15	12	67.63	3.99	11	26.55	1.88	11	5.46	0.86
	16-30	21	68.30	1.39	21	26.94	1.31	21	4.82	0.93
July	1-15	16	67.20	5.99	16	27.86	1.24	16	5.01	0.84
	16-31	19	68.72	2.53	17	26.79	2.31	17	4.46	1.32
Aug.	1-15	7	70.75	4.04	6	24.91	3.78	6	3.94	0.90
	16-31	5	68.96	1.73	5	26.10	2.90	5	4.93	1.40
Sept.	1-15	9	67.36	2.61	9	27.67	2.01	9	5.28	2.30
	16-30	7	68.05	4.76	7	26.45	23.48	7	5.73	2.41

TABLE 22.6. SEASONAL CHANGES IN BODY COMPOSITION OF JUVENILE WESTERN MEADOWLARKS, 1974-1976

		Water (% body weight)			Lean (% body weight)			Fat (% body weight)		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
MALES										
June	1-15	2	69.60	--	2	22.72	--	2	7.69	--
	16-30	5	72.30	1.78	5	23.85	2.16	5	3.92	1.55
July	1-15	6	68.30	9.48	6	27.46	9.52	6	4.24	1.26
	16-30	21	70.09	3.17	21	24.92	2.55	21	4.91	1.43
Aug.	1-15	17	71.62	2.65	14	23.54	2.18	14	4.64	1.09
	16-31	33	70.09	1.92	29	25.46	1.83	29	4.31	0.87
Sept.	1-15	30	69.42	2.66	29	26.81	1.99	29	4.08	0.95
	16-30	27	67.76	2.48	24	26.92	1.46	24	4.95	1.60
Oct.	1-15	11	64.16	3.35	11	27.46	1.47	11	8.38	4.02
FEMALES										
June	16-30	4	70.13	1.49	4	24.80	1.95	4	5.20	1.07
July	1-15	--	--	--	--	--	--	--	--	--
	16-31	13	70.44	1.16	12	23.49	5.27	12	6.03	5.00
Aug.	1-15	13	70.63	3.57	12	24.33	3.99	12	5.00	1.92
	16-31	21	70.41	2.43	18	24.78	1.62	18	4.44	1.03
Sept.	1-15	25	68.64	2.32	23	26.38	1.78	24	4.44	1.18
	16-30	19	67.60	1.46	18	27.30	1.31	18	4.99	1.10
Oct.	1-15	3	62.76	2.81	3	26.71	1.32	3	10.53	4.14
SEX UNDETERMINED										
July	1-15	4	70.54	3.15	4	24.12	2.38	4	5.35	2.61
	16-31	3	70.55	4.82	3	24.99	4.82	3	4.46	0.41
Aug.	1-15	9	72.79	4.83	9	22.83	4.10	9	4.21	1.74
	16-31	5	69.91	1.57	5	24.04	5.55	5	4.04	1.09
Sept.	1-15	5	70.73	1.46	5	25.05	.72	5	4.10	1.02
	16-30	13	67.42	1.37	13	26.51	1.82	13	5.30	1.52
Oct.	1-15	3	64.77	3.06	3	28.20	1.75	3	7.03	4.04

TABLE 22.7. SEASONAL CHANGES IN CARCASS NITROGEN (PERCENT DRY WEIGHT) IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	20	11.6	1.0	--	--	--	--	--	--	--	--	--
	16-30	13	11.3	1.2	3	10.6	1.1	--	--	--	--	--	--
May	1-15	3	10.8	0.5	10	11.4	1.0	--	--	--	--	--	--
	16-31	9	11.6	1.0	5	11.7	1.1	--	--	--	--	--	--
June	1-15	6	11.6	0.7	8	11.5	0.9	1	11.9	--	--	--	--
	16-30	16	11.8	0.7	14	11.6	1.2	4	11.7	1.3	2	11.8	--
July	1-15	13	11.8	0.7	13	11.7	0.9	4	11.7	0.9	1	13.1	--
	16-31	18	12.3	1.8	9	12.1	0.7	16	12.4	1.5	10	10.9	1.6
Aug.	1-15	5	11.8	0.7	6	11.7	0.3	6	11.5	0.8	13	11.8	0.8
	16-31	3	11.7	0.4	1	11.6	--	19	12.2	1.0	9	12.3	1.6
Sept.	1-15	8	12.0	1.0	9	11.5	1.6	27	12.0	0.9	21	11.9	0.8
	16-30	12	11.5	1.1	4	10.4	1.6	21	12.1	1.9	13	11.4	1.3
Oct.	1-15	4	10.0	1.5	--	--	--	7	10.8	1.3	3	9.8	2.2

TABLE 22.8. SEASONAL CHANGES IN PECTORALIS NITROGEN (PERCENT DRY WEIGHT) IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	12	13.4	0.6	--	--	--	--	--	--	--	--	--
	16-30	11	13.3	0.6	4	13.0	0.2	--	--	--	--	--	--
May	1-15	7	13.0	0.1	10	13.1	0.6	--	--	--	--	--	--
	16-31	11	13.1	1.0	12	13.8	2.4	--	--	--	--	--	--
June	1-15	9	13.0	0.8	7	12.9	0.7	--	--	--	--	--	--
	16-30	10	13.5	1.0	7	12.2	1.3	2	13.3	--	1	20.4	--
July	1-15	10	13.0	0.6	9	13.0	0.6	2	13.2	--	1	12.8	--
	16-31	7	13.7	1.8	5	12.6	0.6	8	13.1	0.5	6	12.8	0.6
Aug.	1-15	2	13.6	--	6	12.7	0.4	11	13.7	1.1	8	12.8	1.2
	16-31	3	12.7	0.6	--	--	--	16	12.7	0.8	16	12.9	1.6
Sept.	1-15	5	12.8	0.6	5	12.9	0.9	17	13.0	0.8	24	13.6	2.2
	16-30	7	13.8	0.8	6	12.9	0.8	13	12.7	0.7	10	13.2	0.5
Oct.	1-15	3	12.5	1.2	--	--	--	11	13.3	0.6	3	12.8	0.4

TABLE 22.9. SEASONAL CHANGES IN CALORIES PER GRAM ASH-FREE DRY WEIGHT (CARCASS AND INTEGUMENT) OF WESTERN MEADOWLARKS, 1974

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
July	16-31	1	4707	--	1	4865	--	2	4845	--	4	4922	175
Aug.	1-15	--	--	--	1	4644	--	5	4914	65	4	4851	85
	16-31	1	4783	--	--	--	--	11	4851	114	10	4865	72
Sept.	1-15	1	4853	--	1	5335	--	6	4885	94	13	5001	175
	16-30	--	--	--	2	4897	--	7	5010	243	5	5015	137

TABLE 22.10. SEASONAL CHANGES IN LIVER WET WEIGHT (mg) IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	44	2752.5	691.4	--	--	--	--	--	--	--	--	--
	16-30	31	3051.4	346.6	12	2252.6	285.7	--	--	--	--	--	--
May	1-15	14	2780.0	458.8	26	2681.1	610.3	--	--	--	--	--	--
	16-31	18	2689.0	349.0	16	2662.8	519.4	--	--	--	--	--	--
June	1-15	12	2626.5	438.6	9	2611.1	488.1	2	1954.2	--	--	--	--
	16-30	26	2733.4	343.6	16	2604.6	472.8	5	2640.2	315.5	5	2222.2	300.2
July	1-15	9	2705.2	450.7	7	2580.8	366.2	4	3093.7	388.9	1	2732.1	--
	16-31	17	3218.3	570.4	16	2439.1	369.8	9	3022.1	611.9	13	2724.0	426.0
Aug.	1-15	5	2809.1	436.5	5	2361.9	210.5	13	3332.8	393.9	11	2650.1	377.8
	16-31	5	3572.9	947.4	5	3075.2	386.0	30	3284.7	620.8	18	2635.3	221.8
Sept.	1-15	10	3284.2	422.8	10	2551.4	284.9	36	3316.5	511.7	28	2755.4	479.4
	16-30	13	3313.8	536.6	6	2682.0	296.6	27	3522.4	627.4	19	2789.6	266.7
Oct.	1-15	3	3585.0	780.3	--	--	--	12	3599.8	948.1	2	2799.2	--

A few data were obtained on caloric values of meadowlark tissue (Table 22.9). These values were fairly consistent among themselves but, in general, they fell slightly below those reported previously for birds (Brisbin, 1968), Skar *et al.*, 1972). Constancy in caloric value has usually been found in animal tissues (Richman, 1958; Richman and Slobodkin, 1960; Golley, 1961). These data, and those of other species in this study, support the generalization that nonfat components of migratory birds are fairly homeostatic considering the great seasonal swings that occur in their energy expenditures (see Odum *et al.*, 1965).

Liver

Weights and chemical composition of livers were measured in nearly 600 meadowlarks (Tables 22.10- 22.14). Livers of adult males tended to enlarge

TABLE 22.11. SEASONAL CHANGES IN LIVER COMPOSITION OF ADULT WESTERN MEADOWLARKS, 1974-1976

		Water % liver weight)			Lean (% liver weight)			Fat (% liver weight)		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
Males										
April	1-15	42	70.40	2.42	42	24.67	2.61	42	4.95	2.05
	16-30	26	71.59	4.40	25	24.31	4.06	25	4.14	1.41
May	1-15	14	70.08	1.59	14	25.86	1.24	14	4.06	1.10
	16-31	17	70.51	1.58	17	25.53	1.22	17	3.96	0.69
June	1-15	12	69.69	1.07	12	25.29	2.83	12	4.40	1.05
	16-30	26	70.78	2.06	26	24.32	1.35	26	4.90	1.53
July	1-15	9	70.26	1.98	9	24.57	2.22	9	5.17	0.69
	16-31	17	71.86	1.44	14	24.47	1.33	14	3.59	0.72
Aug.	1-15	6	71.72	1.74	4	23.69	1.75	4	4.21	1.05
	16-31	5	71.59	1.49	4	24.67	2.32	4	3.88	0.68
Sept.	1-15	10	71.93	0.88	8	24.46	0.85	8	3.83	1.01
	16-30	12	70.31	1.49	11	24.58	1.07	11	5.07	1.48
Oct.	1-15	3	68.63	0.57	3	25.55	2.52	3	5.83	2.80
Females										
April	16-30	11	70.35	1.49	11	25.32	1.39	11	4.34	0.45
May	1-15	22	70.28	2.20	21	25.03	1.36	21	5.09	1.39
	16-31	16	70.40	1.72	16	24.85	2.67	16	4.96	1.31
June	1-15	9	71.52	2.55	9	23.42	2.45	9	5.06	0.68
	16-30	15	71.51	2.17	13	23.02	2.87	13	5.28	1.25
July	1-15	9	70.85	2.26	9	24.45	1.69	9	4.81	1.30
	16-31	16	72.07	2.13	16	23.56	2.21	16	4.37	1.10
Aug.	1-15	5	71.94	1.82	4	24.03	1.66	4	4.10	1.31
	16-31	5	71.70	1.72	5	24.14	1.13	5	4.16	1.68
Sept.	1-15	10	71.06	1.40	9	24.88	1.00	9	4.31	0.84
	16-30	8	69.82	1.74	8	25.12	1.43	8	5.46	1.69

TABLE 22.12. SEASONAL CHANGES IN LIVER COMPOSITION OF JUVENILE WESTERN MEADOWLARKS, 1974-1976

		Water % liver weight)			Lean (% liver weight)			Fat (% liver weight)		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
Males										
June	1-15	2	73.27	--	2	23.27	--	2	3.46	--
	16-30	5	71.34	1.26	5	23.88	1.73	5	4.78	0.58
July	1-15	4	72.27	0.19	4	23.62	1.22	4	4.12	1.05
	16-31	17	72.69	1.31	16	23.72	1.36	16	3.64	0.80
Aug.	1-15	13	72.71	2.03	11	24.11	1.62	11	3.73	0.81
	16-31	30	72.06	1.29	28	24.39	1.53	28	3.56	0.98
Sept.	1-15	36	71.56	1.66	34	24.36	1.39	34	4.03	0.86
	16-30	27	69.79	2.87	25	25.32	1.58	25	5.06	2.21
Oct.	1-15	11	68.53	2.19	11	25.12	1.79	11	6.16	3.36
Females										
June	16-30	5	70.12	0.79	5	23.23	3.11	5	6.64	2.94
July	1-15	1	73.54	--	--	21.46	--	1	5.04	--
	16-31	14	72.36	1.15	13	23.40	1.03	13	4.26	1.00
Aug.	1-15	11	71.85	1.92	9	24.85	1.46	9	3.69	1.09
	16-31	18	71.44	1.46	17	25.23	1.30	17	3.31	0.58
Sept.	1-15	28	71.06	1.67	28	24.84	1.44	28	4.11	0.96
	16-30	19	70.22	2.08	18	25.13	1.47	18	4.71	1.68
Oct.	1-15	3	70.72	0.58	2	25.18	--	2	4.43	--

during the last two months of the collection period (Table 22.8). These were matched in size by those of juvenile males. This change in liver size of males was unmatched by seasonal alterations in liver composition; the reported components of the liver remained remarkably constant in terms of relative proportions.

Because of its central role in intermediary metabolism, especially as a storage site for nutrients, the liver may vary considerably in mass according to diet, feeding habits, and energy expenditures (Oakeson, 1953; Hanson, 1962; Pendergast and Boag, 1973; Ankney, 1977).

Of pertinence to the present study, seasonal changes in liver mass have been noted in migrants in that they decrease sharply during spring migration (Oakeson, 1953). Liver weight may also decrease during incubation especially in species, such as geese, that exhibit reduced rates of feeding at that time (Ankney, 1977).

TABLE 22.13. SEASONAL CHANGES IN LIVER DRY LIPID INDEX (FAT AS PERCENT DRY WEIGHT) IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	48	20.32	12.56	--	--	--	--	--	--	--	--	--
	16-30	27	19.19	8.18	11	17.44	1.83	--	--	--	--	--	--
May	1-15	17	15.57	4.58	23	20.45	6.03	--	--	--	--	--	--
	16-31	26	14.60	3.15	18	18.65	5.11	--	--	--	--	--	--
June	1-15	13	16.91	4.70	3	21.68	6.13	2	15.22	--	--	--	--
	16-30	31	19.61	6.36	15	27.91	16.61	5	20.27	4.07	5	30.66	19.59
July	1-15	12	20.97	4.52	10	22.17	7.54	5	18.00	4.84	8	18.73	5.35
	16-31	15	15.17	4.12	20	18.78	6.64	19	16.93	5.14	8	18.21	5.50
Aug.	1-15	5	17.90	4.12	6	18.46	5.12	14	16.32	3.90	12	14.35	2.75
	16-31	4	16.03	4.08	5	17.34	7.11	29	14.69	4.59	21	14.68	4.03
Sept.	1-15	8	15.75	4.60	9	17.38	3.58	34	16.43	3.79	25	17.07	6.05
	16-30	11	20.63	6.45	7	22.09	7.63	24	20.10	8.17	12	17.98	5.24
Oct.	1-15	3	23.71	14.04	--	--	--	10	25.32	18.10	3	23.16	9.64

TABLE 22.14. SEASONAL CHANGES IN LIVER NITROGEN (PERCENT DRY WEIGHT) IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	12	13.0	0.6	--	--	--	--	--	--	--	--	--
	16-30	18	12.9	0.6	6	13.0	0.8	--	--	--	--	--	--
May	1-15	5	12.7	0.5	8	13.1	0.3	--	--	--	--	--	--
	16-31	4	12.7	0.4	3	12.7	0.4	--	--	--	--	--	--
June	1-15	1	12.6	--	2	12.6	--	--	--	--	--	--	--
	16-30	6	12.8	0.4	5	12.9	0.4	1	11.8	--	1	12.9	--
July	1-15	6	12.7	0.5	7	12.9	0.2	1	13.4	--	1	12.7	--
	16-31	5	12.5	0.6	9	12.9	0.7	11	12.7	1.0	4	13.5	0.7
Aug.	1-15	8	13.0	0.8	3	12.6	0.5	7	12.7	0.4	4	12.5	0.2
	16-31	3	12.5	0.3	4	13.1	1.1	13	13.0	0.6	7	13.3	0.5
Sept.	1-15	5	13.0	0.6	5	12.5	0.3	25	13.1	0.7	10	13.0	0.7
	16-30	7	12.9	0.7	4	13.1	0.5	10	13.0	0.6	5	13.0	0.3
Oct.	1-15	1	11.0	--	--	--	--	5	12.5	0.7	2	11.8	--

Other Organs

Other organs of meadowlarks that were weighed routinely included heart (Table 22.15), lungs (Table 22.16), spleen (Table 22.17), adrenal glands (Table 22.18), thyroid glands (Table 22.19), and left kidney (Table 22.20). One kidney was weighed because there is no weight asymmetry between left and right kidneys in birds (Johnson, 1968). Kidneys were also examined for nitrogen content (Table 22.21). Spleens tended to become much larger as the season progressed. Spleens in September were, for example, nearly twice as large as those of April in both adult sexes, a difference that was significant in both cases ($P < 0.05$). This is at least partly related to increased production of blood (and perhaps immune bodies) during molt.

The highly practical aspects of understanding cardiac function has led to its intensive study in a wide range of higher animals. In birds a rather complete review of heart anatomy and physiology has been completed by Sturkie (1976). Heart weights have been reported for many avian species (Hartman, 1955; Brush, 1966). There seems to be a predictable, but allometric relationship between heart size and body size. Hearts are relatively larger in smaller birds. In birds of the approximate size utilized in the present study the heart would be expected to comprise 1 percent or more of body weight. Environmental factors, such as high altitude, are known to affect heart size in birds (see review by Carey and Morton, 1976), but regular seasonal oscillations are unreported. An apparent decrease of heart weight throughout the breeding season in adult female meadowlarks is not readily explanable by us.

Splenic function has been poorly studied in birds, especially wild birds. However, it is known to receive about 3-4 percent of cardiac output in chickens (Sapirstein and Hartman, 1959). And it may vary seasonally in mass in both sedentary (Riddle, 1928b) and migratory (Oakeson, 1953) birds. We find no reported data on breeding birds that can be used for comparative purposes with our data.

Size of adrenal glands varies with species, sex, age, health and a host of factors related to physical stress such as temperature, disease, vitamin deficiency, and exercise (see review by Ringer, 1976a). Daily and seasonal rhythmicities in function are known to occur, particularly in relation to photocycle. Measurement of these functions cannot be determined reliably by whole gland weight; plasma or urinary corticosteroid titers are required (see review by Assenmacher, 1973). Cytologic changes occur seasonally in adrenals of migratory birds (Burger, 1938; Fromme-Bouman, 1962; Lorenzen and Farner, 1964).

The avian thyroid is paired and lobes are located in the neck next to the great blood vessels. As in mammals, the gland is involved in a wide range of functions such as growth, heat production, carbohydrate metabolism, and sexual maturation. It also affects migratory behavior and onset of molt in some birds and growth of young feathers (Assenmacher, 1973).

TABLE 22.15. SEASONAL CHANGES IN HEART WET WEIGHT (mg) IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	50	1560.03	287.80	--	--	--	--	--	--	--	--	--
	16-30	26	1604.48	180.23	12	1257.81	103.50	--	--	--	--	--	--
May	1-15	13	1575.75	204.36	25	1198.23	163.03	--	--	--	--	--	--
	16-31	20	1537.21	137.63	17	1132.81	147.89	--	--	--	--	--	--
June	1-15	14	1393.93	138.43	8	1020.63	80.08	2	749.20	--	--	--	--
	16-30	29	1422.66	189.06	14	1030.04	194.29	6	786.10	176.90	5	773.40	150.50
July	1-15	11	1397.69	197.66	12	1035.21	91.50	4	1176.94	390.60	1	723.00	--
	16-31	18	1381.65	224.40	19	977.65	315.30	20	1138.90	298.40	13	957.20	146.90
Aug.	1-15	7	1511.71	157.49	5	976.44	118.04	17	1169.20	153.40	13	877.10	168.30
	16-31	3	1262.97	82.15	5	999.94	158.75	32	1131.30	141.00	18	898.50	131.10
Sept.	1-15	10	1372.38	197.86	10	975.02	110.40	34	1224.40	171.90	24	972.43	148.80
	16-30	13	1408.00	346.78	8	1067.71	127.30	27	1290.20	151.30	19	1017.30	117.30
Oct.	1-15	3	1793.30	320.03	--	--	--	10	1383.10	77.70	2	1053.90	--

TABLE 22.16. SEASONAL CHANGES IN WET WEIGHT (mg) OF BOTH LUNGS IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	31	1678.7	224.0	--	--	--	--	--	--	--	--	--
	16-30	19	1663.4	227.1	8	1351.7	142.1	--	--	--	--	--	--
May	1-15	6	1801.0	325.3	20	1227.8	143.0	--	--	--	--	--	--
	16-31	13	1601.8	180.2	14	1265.3	172.6	--	--	--	--	--	--
June	1-15	8	1516.7	246.9	8	1198.7	205.0	1	928.4	--	--	--	--
	16-30	19	1584.9	214.1	11	1154.1	238.8	3	1096.8	313.1	3	1013.4	145.0
July	1-15	7	1670.2	289.5	11	1131.0	182.7	2	1555.9	--	1	937.9	--
	16-31	13	1786.5	316.1	13	1254.9	191.2	17	1415.0	196.0	11	1298.7	617.1
Aug.	1-15	4	1551.4	249.9	4	1122.8	64.0	12	1381.8	179.0	11	1046.6	151.7
	16-31	4	1689.4	165.9	4	1245.4	222.7	27	1435.0	190.5	18	1147.6	453.8
Sept.	1-15	8	1585.3	105.3	7	1157.5	119.3	29	1419.5	196.2	21	1127.2	215.1
	16-30	12	1501.6	261.1	5	1158.4	118.6	24	1453.9	225.3	17	1074.0	156.2
Oct.	1-15	3	1621.4	286.6	--	--	--	8	1453.2	330.4	3	1064.7	30.4

TABLE 22.17. SEASONAL CHANGES IN SPLEEN WET WEIGHT (mg) IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	47	141.7	111.1	1	109.4	--	--	--	--	--	--	--
	16-30	29	121.0	94.3	14	137.1	104.0	--	--	--	--	--	--
May	1-15	36	130.3	86.2	28	152.1	128.0	--	--	--	--	--	--
	16-31	32	174.7	209.6	30	136.9	98.7	--	--	--	--	--	--
June	1-15	30	120.8	100.8	22	117.2	74.7	--	--	--	--	--	--
	16-30	41	180.8	131.0	13	141.5	81.1	13	129.5	126.6	16	77.9	34.8
July	1-15	20	180.0	167.0	17	141.8	105.0	15	70.3	31.2	8	70.0	29.2
	16-31	21	243.4	121.1	18	152.8	101.3	25	130.3	104.2	31	178.9	254.1
Aug.	1-15	16	268.8	238.5	9	134.5	86.8	18	218.2	208.3	11	193.7	198.0
	16-31	6	446.1	467.9	9	173.1	103.4	25	182.8	199.3	14	156.8	158.6
Sept.	1-15	7	235.7	130.2	10	191.5	67.6	28	178.6	175.8	25	138. 5	71.6
	16-30	11	287.5	165.3	8	221.7	100.0	24	140.7	85.5	19	178.7	138.9
Oct.	1-15	1	164.9	--	0	--	--	11	201.7	115.5	4	162.6	102.3

TABLE 22.18. SEASONAL CHANGES IN WET WEIGHTS (mg) OF PAIRED ADRENAL GLANDS IN WESTERN MEADOWLARKS, 1974-1978

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	34	7.2	2.8	--	--	--	--	--	--	--	--	--
	16-30	23	7.6	3.4	9	5.9	2.9	--	--	--	--	--	--
May	1-15	31	6.2	2.1	27	6.4	3.3	--	--	--	--	--	--
	16-31	28	6.3	2.4	24	6.0	2.6	--	--	--	--	--	--
June	1-15	34	7.1	3.1	21	6.5	2.7	1	7.2	--	--	--	--
	16-30	43	6.7	2.8	13	7.1	3.4	11	7.1	2.1	16	5.7	2.4
July	1-15	20	7.0	2.7	19	6.4	2.0	12	6.2	3.2	8	4.3	2.0
	16-31	19	6.8	2.1	18	5.1	2.0	27	6.9	2.2	31	6.0	2.9
Aug.	1-15	10	6.9	1.6	10	6.9	2.9	15	6.9	2.8	14	5.7	1.9
	16-31	5	6.0	1.6	6	5.3	1.3	26	5.9	2.3	15	5.6	1.5
Sept.	1-15	8	6.3	3.0	6	7.0	2.4	32	7.3	3.0	19	7.3	3.1
	16-30	10	7.4	1.7	8	8.9	3.5	21	8.0	2.4	15	6.2	2.1
Oct.	1-15	1	7.9	--	--	--	--	8	7.8	2.1	3	9.3	2.9

TABLE 22.19. SEASONAL CHANGES IN WET WEIGHTS (mg) OF PAIRED THYROID GLANDS IN WESTERN MEADOWLARKS, 1974-1978

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	39	6.4	2.6	1	5.0	--	--	--	--	--	--	--
	16-30	23	6.1	2.0	7	7.3	2.3	--	--	--	--	--	--
May	1-15	33	6.3	2.7	23	8.1	6.2	--	--	--	--	--	--
	16-31	29	8.5	3.6	16	8.5	4.3	--	--	--	--	--	--
June	1-15	30	7.4	3.1	18	8.0	4.8	2	11.7	--	--	--	--
	16-30	40	9.7	4.8	16	8.5	4.3	12	8.4	4.4	15	7.3	4.8
July	1-15	17	8.7	3.8	18	6.6	3.9	13	5.2	1.4	7	5.2	1.3
	16-31	19	8.8	3.1	18	7.3	3.1	23	7.6	2.9	25	5.3	2.1
Aug.	1-15	14	8.4	2.9	8	7.5	2.2	10	6.9	2.2	6	7.3	2.7
	16-31	5	8.2	3.4	10	5.7	2.2	14	7.7	2.3	5	5.8	1.2
Sept.	1-15	5	7.0	3.4	4	7.7	2.7	21	8.2	2.0	8	7.7	3.2
	16-30	5	8.5	3.5	2	3.3	--	11	8.3	2.2	5	8.7	2.2
Oct.	1-15	--	--	--	--	--	--	--	--	--	1	11.6	--

TABLE 22.20. SEASONAL CHANGES IN WET WEIGHTS (mg) OF LEFT KIDNEY IN WESTERN MEADOWLARKS, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	29	385.5	127.7	--	--	--	--	--	--	--	--	--
	16-30	17	502.9	58.3	10	434.7	43.5	--	--	--	--	--	--
May	1-15	9	478.4	45.6	22	460.6	85.2	--	--	--	--	--	--
	16-31	16	471.6	43.2	18	443.9	55.8	--	--	--	--	--	--
June	1-15	11	464.3	61.6	9	424.4	70.0	1	343.3	--	--	--	--
	16-30	26	487.5	54.9	13	449.9	45.9	5	409.1	66.4	2	377.0	--
July	1-15	7	479.2	68.9	7	458.0	46.4	5	385.2	40.8	1	282.6	--
	16-31	15	569.8	99.0	13	437.2	51.2	15	500.2	79.7	11	423.8	57.8
Aug.	1-15	5	546.8	50.4	4	409.2	52.7	10	471.4	109.9	11	415.8	63.6
	16-31	3	595.9	77.3	4	541.6	101.5	28	478.8	133.6	18	419.7	80.8
Sept.	1-15	9	592.6	63.5	8	463.0	49.3	33	542.9	86.3	22	449.1	57.8
	16-30	7	517.6	69.0	7	468.1	60.6	25	512.2	86.9	17	464.3	41.5
Oct.	1-15	3	617.8	90.7	--	--	--	10	603.8	47.3	2	464.7	--

TABLE 22.21. SEASONAL CHANGES IN KIDNEY NITROGEN (PERCENT DRY WEIGHT) IN WESTERN MEADOWLARKS, 1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	13	12.3	9.3	--	--	--	--	--	--	--	--	--
	16-30	6	12.5	1.9	4	12.4	5.2	--	--	--	--	--	--
May	1-15	4	12.3	2.2	8	12.3	10.4	--	--	--	--	--	--
	16-31	9	12.3	4.2	4	12.4	6.0	--	--	--	--	--	--
June	1-15	7	12.0	2.4	5	12.2	5.1	1	12.0	--	--	--	--
	16-30	17	12.3	5.5	9	12.0	1.7	3	11.8	0.6	2	12.5	--
July	1-15	4	12.4	0.8	4	12.1	1.0	3	12.0	0.6	--	--	--
	16-31	11	12.4	4.8	8	12.7	14.5	11	12.4	3.9	5	12.3	4.3
Aug.	1-15	5	12.5	7.7	1	11.8	--	3	12.4	2.5	2	13.9	--
	16-31	1	13.9	--	4	13.9	6.5	13	13.9	13.1	2	13.3	--
Sept.	1-15	3	13.6	4.2	3	13.1	1.5	4	14. 2	15.3	3	14.1	18.2
	16-30	2	15.6	--	--	--	--	6	15.1	16.3	4	16.1	21.75

TABLE 22.22. SEASONAL CHANGES IN DIAMETER (mm) OF LARGEST THYMUS LOBE IN WESTERN MEADOWLARKS, 1974-1975

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	22	0.4	0.8	--	--	--	--	--	--	--	--	--
	16-30	15	2.5	1.2	6	2.8	0.9	--	--	--	--	--	--
May	1-15	7	3.0	0.7	14	3.5	0.9	--	--	--	--	--	--
	16-31	11	3.6	0.7	14	3.8	0.6	--	--	--	--	--	--
June	1-15	9	3.6	1.1	7	4.1	1.2	--	--	--	--	--	--
	16-30	8	3.9	1.0	8	3.6	1.4	1	6.8	--	1	4.2	--
July	1-15	5	5.3	1.6	6	4.1	1.6	2	5.5	--	1	4.6	--
	16-31	6	4.4	1.3	5	4.1	1.1	6	5.5	1.2	6	6.1	1.3
Aug.	1-15	1	4.4	--	4	4.8	1.7	9	5.7	1.4	5	6.0	1.5
	16-31	3	6.7	1.4	--	--	--	15	5.6	1.1	14	5.9	1.1
Sept.	1-15	4	5.9	2.8	4	5.1	0.5	14	5.3	0.7	18	5.0	1.2
	16-30	5	5.7	1.1	4	5.6	1.2	12	5.2	1.2	10	5.0	0.8

Weight of thyroid glands varies seasonally, being largest in winter in most, (see Miller, 1939; Wilson and Farner, 1960), but not all, species. Increased size can be a function of both cell size or number (see reviews by Höhn, 1950 and Ringer, 1976b). Thyroid (and adrenal) weights sometimes vary inexplicably in wild birds (Hartman, 1946). Some of this variance may be related to shifts in diet (Riddle and Fisher, 1926) as well as in temperature. There is, however, a general positive correlation of both glands with body size (Hartman and Brownell, 1961). And within a given species, seasonal changes in weight of thyroids may serve as a useful index to secretory activity (Kendeigh and Wallin, 1966). The perspective provided by histological examination is necessary to understand functional condition of thyroids at a given time; weights alone are unsatisfactory for this purpose.

TABLE 22.23. SEASONAL CHANGES IN WET WEIGHT (mg) OF BURSA OF FABRICIUS IN JUVENILE WESTERN MEADOWLARKS, 1974-1976

	Males			Females		
	N	Mean	S.D.	N	Mean	S.D.
June 1-15	1	51.5	--	--	--	--
16-30	16	99.5	34.1	14	83.8	36.1
July 1-15	14	98.8	34.8	7	121.7	41.0
16-31	30	170.1	71.0	29	152.6	66.0
Aug. 1-15	21	173.4	63.9	14	156.1	76.7
16-31	33	186.1	54.7	18	171.2	29.5
Sept. 1-15	34	196.2	72.2	24	170.8	60.5
16-30	24	192.2	60.3	19	158.6	39.5
Oct. 1-15	10	160.6	47.4	2	169.7	--

The kidneys of birds are symmetrically paired structures located in bony depressions of the fused pelvis. They usually comprise about 1 percent of the body weight in birds. Their relationship to body weight is allometric, however, and they are relatively larger in small birds and in some desert and marine types. The kidneys through osmoregulatory controls, maintain water and electrolyte balance (see review by Shoemaker, 1972).

Data were also gathered on the diameter of the largest thymus lobe (Table 22.22), weight of the bursa of Fabricius (Table 22.23), and gizzard weight (Table 22.24). The bursa of Fabricius is a dorsal diverticulum of the

TABLE 22.24. SEASONAL CHANGES IN EMPTY GIZZARD WET WEIGHT (mg) IN WESTERN MEADOWLARK, 1974-1976

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	47	2945.6	390.5	--	--	--	--	--	--	--	--	--
	16-30	29	2403.3	471.9	11	1010.9	207.1	--	--	--	--	--	--
May	1-15	17	2145.5	222.1	26	1811.6	238.0	--	--	--	--	--	--
	16-31	24	1957.9	306.9	20	1817.5	237.4	--	--	--	--	--	--
June	1-15	16	1803.1	330.9	14	1707.5	218.0	1	1955.6	--	--	--	--
	16-30	34	1867.8	382.8	21	1548.6	179.4	5	1726.8	273.1.	5	1459.4	127.9
July	1-15	15	1845.9	220.6	11	1520.6	154.6	5	1746.0	379.5	1	1465.2	--
	16-31	21	2021.7	406.8	17	1608.8	203.9	20	1984.8	212.6	13	1828.1	178.0
Aug.	1-15	8	2353.8	243.4	8	1839.7	354.0	16	2512.6	316.2	12	1969.6	292.0
	16-31	5	2716.1	285.2	5	2265.8	326.8	32	2700.6	386.0	18	2200.0	194.8
Sept.	1-15	10	2720.2	221.4	10	2283.4	146.1	35	2908.3	459.8	26	2424.8	319.0
	16-30	11	3135.8	369.9	7	2411.4	192.2	27	3060.6	303.7	18	2548.1	279.9
Oct.	1-15	3	3453.5	216.4	--	--	--	11	3140.3	266.4	1	2774.7	--

proctodeal region of the cloaca. Its major functions include the regulation of humoral antibody production (Glick, 1964; Chang *et al.*, 1957; Warner and Szenberg, 1964; Cooper *et al.*, 1967). The secretion of a diffusible factor which acts on lymphoid tissue (Glick, 1960a; Jankovich and Leskovitz, 1965; St. Pierre and Ackermann 1965), and the synthesis of immunoglobulins (Grossi *et al.*, 1968; Thornbecke *et al.*, 1968; Zaccheo *et al.*, 1968; Glick, 1977). Basically it contributes to immunological competence.

The bursa is restricted to birds, being largest in young birds and tending to involute with advancing age. Maximum size is reached during the first few weeks of life, but the exact time varies among species, being about 69 days in the pigeon (Riddle, 1928a), 110 days in the pheasant (Kirkpatrick 1944) and 56 days in the mallard (Johnson 1961). After the bursa obtains maximum size it involutes and eventually disappears (Jolly, 1913; Schauder, 1923, Glick, 1960b; Ward and Middleton, 1971).

Both the thymus and the bursa of the western meadowlark persist for an as yet undefined period following the assumption of the winter plumage. This allows us to easily distinguish adult birds and birds of the year at least throughout the fall.

The bursa is necessary for the development of antibody-mediated responses. It normally involutes when a bird reaches sexual maturity. However, it will involute earlier if the younger bird is subjected to stress. It will, for example, regress in the presence of glucocorticoids (see Lewis *et al.*, 1976). Consequently, it should be very useful for identifying stressors of young birds. Sudden regressive changes in the structure of the bursa are more likely to be translated quickly into histological changes than into reductions in the weight of the gland.

The thymus gland is an elongate structure, usually with seven lobes, that lies laterally in the mid- and lower neck. The avian thymus, like that of mammals, is involved in immunological processes, including lymphocyte formation. Along with the bursa it is thought to comprise a dual immunologic system in birds. Both organs direct the maturation of immunologically competent cells capable of reacting to antigens (Assenmacher, 1973).

In all vertebrates the thymus typically increases in size until sexual maturity and then regresses markedly. In birds, however, the process can be reversed and the gland may enlarge again following reproduction (Höhn, 1956). This was observed in meadowlarks in that juveniles had relatively large glands whereas those of adults were small during the first half of the summer (the period of reproduction) and tended to enlarge thereafter (Table 22.22).

The gizzard or stomach of seed-eating birds characteristically is highly muscular in keeping with its function as the site where food is pulverized before passage to the main digestive and absorptive portions of the gastrointestinal tract. In this capacity it is, of course, the functional analog of teeth in birds. It may also serve as a chamber for food storage and for acid proteolysis (Ziswiler and Farner, 1972).

Seasonal changes in gizzard size are to be anticipated as a result of dietary changes; both due to food choice or to quantity of intake. Reduced

TABLE 22.25. PERCENT OF WESTERN MEADOWLARKS SHOWING MOLT, 1974-1977

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Number in molt	Percent molting	N	Nunber in molt	Percent molting	N	Number in molt	Percent molting	N	Number in molt	Percent molting
April	1-15	76	0	--	1	0	--	--	--	--	--	--	--
	16-30	49	0	--	15	0	--	--	--	--	--	--	--
May	1-15	47	0	--	45	0	--	--	--	--	--	--	--
	16-31	46	0	--	36	0	--	2	0	0	--	--	--
June	1-15	42	0	--	26	0	--	2	0	0	1	0	0
	16-30	51	1	1.96	29	0	--	8	1	12.50	8	2	25.00
July	1-15	27	11	40.74	28	5	17.86	19	7	36.84	7	5	71.43
	16-31	28	23	82.14	24	10	41.67	30	23	76.67	33	17	51.52
Aug.	1-15	14	13	92.86	12	9	75.00	33	28	84.85	25	23	92.00
	16-31	7	5	71.43	13	13	100.00	45	44	97.78	44	31	70.45
Sept.	1-15	12	12	100.00	17	16	94.12	52	52	100.00	38	36	94.74
	16-30	14	13	92.85	8	7	87.50	28	25	89.29	22	22	100.00
Oct.	1-15	3	1	33.33	--	--	--	11	9	81.82	3	2	66.67

TABLE 22.26. SEASONAL CHANGES OF INTEGUMENT DRY WEIGHT (mg) IN WESTERN MEADOWLARKS, 1974-1977

		Adult Males			Adult Females			Juvenile Males			Juvenile Females		
		N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
April	1-15	49	10001.0	1211.6	--	--	--	--	--	--	--	--	--
	16-30	35	9397.0	809.6	13	7800.0	795.8	--	--	--	--	--	--
May	1-15	19	8822.2	582.9	23	7639.9	1116.4	--	--	--	--	--	--
	16-31	27	8844.4	799.9	22	7145.9	785.2	--	--	--	--	--	--
June	1-15	17	8548.3	681.6	15	6671.3	752.3	2	6336.2	--	--	--	--
	16-30	34	8037.0	774.1	22	6235.2	538.1	5	4366.9	741.6	1	4461.6	--
July	1-15	16	7788.4	855.6	15	6317.4	693.9	6	4815.7	450.6	1	3591.5	--
	16-31	22	7228.2	648.2	20	5778.0	753.5	21	4903.4	369.9	14	4117.3	562.9
Aug.	1-15	8	7181.1	766.3	9	5946.0	1192.7	19	5259.8	715.7	12	4624.8	1082.6
	16-31	4	8187.1	562.4	5	6680.8	1708.8	32	6044.8	1552.8	20	4909.6	751.4
Sept.	1-15	10	9043.4	1202.2	9	7201.4	1254.8	38	7858.5	1456.8	30	6611.8	1665.2
	16-30	12	10119.0	780.5	7	7634.7	864.7	28	8866.6	1530.8	19	7455.0	1228.7
Oct.	1-15	3	11913.7	1613.9	--	--	--	11	9955.1	841.8	4	7964.7	717.8

feeding results in decreased gizzard size and increased feeding, as in premigratory hyperphagia, results in hypertrophy (Breitenbach *et al.*, 1963; Anderson, 1972; Moss, 1974; Ankney, 1977).

Gizzard weight decreased significantly from April to May in adult male meadowlarks ($P < 0.05$). It then increased substantially during the last two months of the season ($P < 0.01$). Contents of the gizzards of our specimens were also weighed and categorized at 3-hr intervals throughout the day. No diurnal feeding pattern was apparent. Uncertainties regarding rate of food passage in the gut make these data difficult to evaluate.

Molt

Postnuptial molt in the population lasted from July through the end of collecting and tended to begin earlier in males than in females (Table 22.25). A complete molt involving flight feathers occurred also in juveniles and their molt had approximately the same tempo as that of adults (Table 22.25). Weights of the dry integument increased toward the end of the season as new, unworn feathers replaced the old (Table 22.26).

Molt and reproduction in most temperate zone migrants do not overlap in time, a relationship that seems to hold for all species in the present study (Lewis, Morton and Kern; unpublished data).

Lean body mass was relatively stable during molt in our meadowlarks, a phenomenon also observed in chaffinches (*Fringilla coelebs*) by Gavrilov and Dolnik (1974), in European tree sparrows (*Passer m.. montanus*) by Myrcha and Pinowski (1970), and white-crowned sparrows (*Zonotrichia leucophrys gambelii*) by Chilgren (1977). Such stability suggests that feather growth was not achieved at the expense of body protein.

We analyzed data on body lipid as a function of molt stage to better appreciate the relationship of molt to onset of premigratory fattening. Results show that as the molt comes to a close, meadowlarks begin to fatten (Table 22.27). Birds having completed or nearly completed molt (category 0-1) had significantly more body lipid than those still growing two or more pairs of remiges ($P < 0.01$). Thus molt within the population was not perfectly synchronous.

Slight increases in body weight during postnuptial molt have been documented for a number of migratory species, usually this was attributed to the new plumage. In at least one case, however, a major increase in weight was due to fattening (Morton and Welton, 1973).

REFERENCES

Adolph, E. F. and F. W. Hegeness. 1971. Age Changes in Body Water and Fat in Fetal and Infant Mammals. Growth, 35:55-63.

TABLE 22.27. BODY LIPID AS PERCENT OF BODY WEIGHT IN WESTERN MEADOWLARKS DURING AND AT END OF MOLT

	Adult Males			Adult Females			Juvenile Males			Juvenile Females			All Birds		
	6+	2-5	0-1	6+	2-5	0-1	6+	2-5	0-1	6+*	2-5	0-1	6+	2-5	0-1
N	6	10	5	11	9	2	36	13	8	22	13	3	75	45	18
Mean	3.91	5.65	9.92	4.13	6.33	8.51	4.31	5.73	9.49	4.65	5.18	12.69	4.36	5.67	10.03
S.D.	0.92	0.94	3.08	0.75	1.65	--	0.96	2.61	3.49	1.58	0.87	0.50	1.15	1.72	3.10

* Category 6+ means six or more pairs of remiges still had to grow in, 2-5 means two to five pairs miges of remiges still had to grow in, and 0-1 means only one pair of remiges still had to grow in or molt had been completed.

Aleksiuk, M. and K. W. Stewart. 1971. Seasonal Changes in the Body Composition of the Garter Snake (*Thamophis sirtalis parietalis*) at Northern Latitudes. Ecology, 52:485490.

Anderson, W. L. 1972. Dynamics of Condition Parameter and Organ Measurements in Pheasants. Illinois Nat. Hist. Surv. Bull., 30:455-497.

Ankney, C. D. 1977. Feeding and Digestive Organ Size in Breeding Lesser Snow Geese. Auk, 94:275-282.

Assenmacher, I. 1973. The Peripheral Endocrine Glands. In: D. S. Farner and J. R. King eds. Avian Biology, Vol. 3. Academic Press, New York. pp. 183-286.

Baldwin, P. S. and C. Kendeigh. 1938. Variations in the Weight of Birds. Auk, 55:416-467.

Baldwin, S. P., H. C. Oberholser and L. G. Worley. 1931. Measurements of Birds. Sci. Publ. Cleveland, Mus. Nat. Hist., 2:1-165.

Breitenbach, R. P., C. L. Nagra and R. K. Meyer. 1963. Effect of Limited Food Intake on Cyclic Annual Changes in Ring-Necked Pheasant Hens. J. Wildl. Mgmt., 27:24-36.

Brisbin, I. L., Jr. 1968. A Determination of the Caloric Density and Major Body Components of Large Birds. Ecology, 49:792-794.

Brush, A. H. 1966. Avian Heart Size and Cardiovascular Performance. Auk, 83:266-273.

Burger, J. W. 1938. Cyclic Changes in the Thyroid and Adrenal Cortex of the Male Starling, *Sturnus vulgaris* , and Their Relation to the Sexual Cycle. Amer. Nat., 72:562-570.

Cameron, E. S. 1907. The Birds of Custer and Dawson Counties, Montana. Auk, 24:389-406.

Carey, C. and M. I. Morton. 1976. Aspects of Circulatory Physiology of Montane and Lowland Birds. Comp. Biochem. Physiol., 54A:51-74.

Chang, T. S., M. S. Rheins and A. R. Winter. 1957. The Significance of the Bursa of Fabricius in Antibody Production in Chickens. Poultry Sci., 36:735-738.

Chilgren, J. D. 1977. Body Composition of Captive White-Crowned Sparrows during Postnuptial Molt. Auk, 94:677-688.

Clark, G. A., Jr. 1979. Body Weights of Birds: A Review. Condor, 81:193-202.

Connell, C., E. Odum and H. Kale. 1960. Fat-Free Weights of Birds. Auk, 77:1-9.

Cooper, M. D., A. E. Gabrielsen and R. A. Good. 1967. Role of the Bursa of Fabricius and Other Central Lymphoid Tissues in Immunological Disease. Ann. Rec. Med., 18:113-138.

Fehrenbacher, L. H. and E. D. Fleharty. 1976. Body Composition, Energy Content, and Lipid Cycles of Two Species of Pocket Gophers (*Geomys bursarius* and *Pappagaomys castanops*) in Kansas. Southwestern Nat., 21:185-198.

Fromme-Bouman, H. 1962. Jahresperiodische Untersuchungen and der Nebennierenrinde der Amsel (*Turdus merula* L.). Vogelwarte, 21:188-198.

Gavrilov, V. M. and V. R. Dolnik. 1974. Bioenergetics and Regulations of the Postnuptial and Postjuvenal Moult in Chaffinches (*Fringilla coelebs coelebs* L.). Akademiya Nauk SSR Trudy Zoologicheskogo Institute Tom Lv. pp. 14-61.

Glick, B. 1960a. Extracts From the Bursa of Fabricius a Lymphoepithelial Gland of the Chicken, Stimulate the Production of Antibodies in Bursectomized Birds. Poultry Sci., 39:1097-1101.

Glick, B. 1960b. Growth of Bursa of Fabricius and its Relationship to the Adrenal Gland in the White Pekin Duck, White Leghorn, Outbred and Inbred New Hampshire. Poultry Sci., 39:130-139.

Glick, B. 1964. The Bursa of Fabricius and the Development of Immunologic Competence. In: R. A. Good and A. E. Gabrielsen, eds. Thymus in Immunobiology. Hoeber-Harper, New York. pp. 343-353.

Glick, B. 1977. The Bursa of Fabricius and Immunoglobulin Synthesis. Int. Rev. Cytology, 48:345-402.

Golley, F. B. 1961. Energy Values of Ecological Materials. Ecology, 42:581-584.

Gross, A. O. 1958. Western Meadowlark. In: A. C. Bent, eds. Life Histories of North American Blackbirds, Orioles, Tanagers, and Allies. U.S. Nat. Mus. Bull., 211-84-98.

Grossi, C. E., V. Genta, M. Ferrarini and D. Zaccheo. 1968. Localization of Immunoglobulins in the Developing Chicken Bursa of Fabricius. Rev. Franc. et Clin. Biolog., 13:497-500.

Hanson, H. C. 1962. The Dynamics of Condition Factors in Canada Geese and Their Relation to Seasonal Stresses. Arctic Inst. N. Amer. Tech. Paper No. 12.

Hartman, F. A. 1946. Adrenal and Thyroid Weights in Birds. Auk, 63:42-63.

Hartman, F. A. 1955. Heart Weight in Birds. Condor, 57:221-238.

Hartman, F. A. and K. A. Brownell. 1961. Adrenal and Thyroid Weights in Birds. Auk, 78:397-422.

Helms, C. W. 1968. Food, Fat, and Feathers. Amer. Zool., 8:151-167.

Helms, C. W. and R. B. Smythe. 1969. Variation in Major Body Components of the Tree Sparrow (*Spizella arboreo*) Sampled Within the Winter Range. Wilson Bull., 81:280-292.

Höhn, E. O. 1950. Physiology of the Thyroid Gland in Birds: a Review. Ibis, 92:464-473.

Höhn, E. O. 1956. Seasonal Recrudescence of the Thymus in Sexually Mature Birds. Can. J. Biochem. Physiol., 34:90-101.

Jankovic, B. D. and S. Leskowitz. 1965. Restoration of Antibody Producing Capacity in Bursectomized Chickens by Bursal Crafts in Millipore Chambers. Proc. Soc. Exp. Biol. Med., 118:1164-1166.

Johnson, O. W. 1961. Reproductive Cycle of the Mallard Duck. Condor, 63:351-364.

Johnson, O. W. 1968. Some Morphological Features of Avian Kidneys. Auk, 85:216-228.

Jolly, J. 1913. L'involution Physiologique de la Bourse de Fabricius et Ses Relations avec l'apparation de la Maturite Sexuelle. C. R. Soc. Biol., 75:638-648.

Kendeigh, C. S. and H. E. Wallin. 1966. Seasonal and Taxonomic Differences in the Size and Activity of the Thyroid Glands in Birds. Ohio J. Sci., 66:369-379.

Kirkpatrick, C. M. 1944. The Bursa of Fabricius in Ring-Necked Pheasants. J. Wildl. Mgmt., 8:118-129.

Krulin, G. S. and J. A. Sealander. 1972. Annual Lipid Cycle of the Gray Bat, *Myatis grisescens*. Comp. Biochem, Physiol., 42:537-549.

Lanyon, W. E. 1956. Ecological Aspects of the Sympatric Distribution of Meadowlarks in the Northcentral States. Ecology, 37:98-108.

Lanyon, W. E. 1962. Specific Limits and Distribution of Meadowlarks in Desert Grassland. Auk, 79:183-207.

Lewis, R. A. and M. L. Morton. 1976. Effects of Coal-Fired Power Plant Emissions on Animals: a Summary. In: R. A. Lewis, N. R. Glass and A. S. Lefohn, eds. The Bioenvironmental Impact of Coal-fired Power Plant, Second Interim Report, Colstrip, Montana. EPA-600/3-76-013. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 140-187.

Lewis, R. A., M. L. Morton and S. C. Jones. 1976. The Effects of Coal-Fired Power Plant Emissions on Vertebrate Animals in Southeastern Montana. In: R. A. Lewis, N. R. Glass and A. S. Lefohn, eds. The Bioenvironmental Impact of Coal-fired Power Plant, Second Interim Report, Colstrip, Montana. EPA-600/3-76-013. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 130-177.

Lewis, R. A., M. L. Morton, M. D. Kern, J. Chilgren and E. M. Preston. 1978. The Effects of Coal-Fired Power Plant Emissions on Vertebrate Animals in Southeastern Montana. In: E. M. Preston and R. A. Lewis eds. The Bioenvironmental Impact of a Coal-fired Power Plant, Third Interim Report, Colstrip, Montana. EPA-600/3-78-021. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 213-279.

Lofts, B. and R. K. Murton. 1973. Reproduction in Birds. In: D. S. Farner and J. R. King, eds. Avian Biology, Vol. 3. Academic Press, New York. pp. 1-107.

Lorenzen, L. and D. S. Farner. 1964. An Annual Cycle in the Interrenal Tissue of the Adrenal Gland of the White-Crowned Sparrow, *Zonotrichia leucophrys gambelii* . Gen. Comp. Endocrinol., 4:253-263.

McNeil, R. 1969. Le Determenation du Contenu Lipidque et de la Capacite' de Vol Chez Quelques Especes D;oiseaux de Rivage (Charadriidae et Scolopacidae). Can. J. Zool., 47:525-536.

Mewaldt, L. R. and J. R. King. 1977. The Annual Cycle of White-Crowned Sparrows *Zonotrichia leucophrys nuttalli* in Coastal California. Condor, 79:445-455.

Miller, D. S. 1939. A Study of the Physiology of the Sparrow Thyroid. J. Expt. Zool., 80:259-284.

Morton, M. L. 1975. Seasonal Cycles of Body Weights and Lipids in Belding Ground Squirrels. Bull. So. Calif. Acad. Sci., 74:128-143.

Morton, M. L. 1976. Adaptive Strategies of *Zonotrichia* Breeding at High Latitude or High Altitude. Proc. 16th Intern. Ornithol. Congr., Canberra, pp. 322-336.

Morton, M. L., J. R. King and D. S. Farner. 1969. Postnuptial and Postjuvenal Molt in White-Crowned Sparrows in Central Alaska. Condor, 71:376-385.

Morton, M. L., C. S. Maxwell and C. E. Wade. 1974. Body Size, Body Composition, and Behavior of Juvenile Belding Ground Squirrels. Great Basin Nat., 34:121-134.

Morton, M. L. and D. E. Welton. 1973. Postnuptial Molt and its Relation to Reproductive Cycle and Body Weight in Mountain White-Crowned Sparrows (*Zonotrichia leucophrys oriantha*). Condor, 75:184-189.

Moss, R. 1974. Winter Diets, Gut Lengths and Interspecific Competition in Alaska Ptarmigan. Auk, 91:737-746.

Myrcha, A. and J. Pinowski. 1970. Weights, Body Composition, and Caloric Value of Postjuvenal Molting European Tree Sparrows *(Passer montanus)*. Condor, 72:175-181.

Oakeson, B. B. 1953. Cyclic Changes in Liver and Spleen Weights in Migratory White-Crowned Sparrows. Condor, 55:3-16.

Odum, E. P., S. G. Marshall and T. G. Marples. 1965. The Caloric Content of Migrating Birds. Ecology, 46:901-904.

Pendergast, B. A. and D. A. Boag. 1973. Seasonal Changes in the Internal Anatomy of Spruce Grouse in Alberta. Auk, 90:307-317.

Perkins, R. J. and M. D. Dahlberg. 1971. Fat Cycles and Condition Facts of Altamaha River Shads. Ecology, 52:359-362.

Richman, S. 1958. The Transformation of Energy by *Daphnia pulex.* Ecological Monogr., 28:273-291.

Richman, S. and B. Slobodkin. 1960. A Micro-Bomb Calorimeter for Ecology. Ecology, 41:83-89.

Riddle, O. 1928a. Growth of the Gonads and Bursa Fabricii in Doves and Pigeons with Data for Body Growth and Age at Maturity. Amer. J. Physiol., 86:248-265.

Riddle, O. 1928b. Sex and Seasonal Differences in Weight of Liver and Spleen. Proc. Soc. Exp. Biol. and Med., 25:474-476.

Riddle, O. and W. S. Fisher. 1926. Seasonal Variation of Thyroid Size in Pigeons. Ibis, 92:464-487.

Ringer, R. K. 1976a. Adrenals. In: P. D. Sturkie, ed. Avian Physiology. Springer-Verlag, New York. pp. 372-382.

Ringer, R. K. 1976b. Thyroids. In: D. Sturkie, ed. Avian Physiology. Springer-Verlag, New York. pp. 348-371.

Rohwer, S. A. 1973. Significance of Sympatry to Behavior and Evolution of Great Plains Meadowlarks. Evolution, 27:44-57.

Sapirstein, L. A. and F. A. Hartman. 1959. Cardiac Output and its Distribution in the Chicken. Amer. J. Physiol., 196:75-752.

Schauder, W. 1923. Martin P. Lehrbuch der Anatomie de Naustiere. Anat. der Hausvogel, 4:339-398. Schickardt and Ebner, Stuttgart, Germany.

Shoemaker, V. H. 1972. Osmoregulation and Excretion of Birds. In: D. S. Farner and J. R. King, eds. Avian Biology, Vol. 2. Academic Press, New York. pp. 527-574.

Skar, H. J., A. Hagen and E. Ostbye. 1972. Caloric Values, Weight, Ash and Water Content of the Body of the Meadow Pipit (*Anthus practenis* L.). Norweg. J. Zool., 20:51-59.

St. Pierre, R. L. and G. A. Ackerman. 1965. Bursa of Fabricius in Chicken Possible Humoral Factor. Science. 147:1307-1308.

Sturkie, P. D. 1976. Heart and Circulation: Anatomy, Hemodynamics, Blood Pressure, Blood Flow, and Body Fluids. In: P. D. Sturkie, ed. Avian Physiology. Springer-Verlag, New York. pp. 76-101.

Sturkie, P. D. and Mueller, W. J. 1976. Reproduction in the Female and Egg Production. In: P. D. Sturkie, ed. Avian Physiology. Springer-Verlag, New York. pp. 303-330.

Telford, S. R., Jr. 1970. Seasonal Fluctuations in Liver and Fat Body Weights of the Japanese Lactertid *Takydromas tachydromoides* Schlegel. Copeia, 1970:681-688.

Thornbecke, G. J., N. L. Warner, G. M. Hochwald and S. H. Ohanian. 1968. Immune Globulin Production by the Bursa of Fabricius in Young Chickens. Immunology, 15:123-134.

Ward, J. G. and A. I. A. Middleton. 1971. Weight and Histological Studies of Growth and Regression in the Bursa of Fabricius in Mallard, *Anas platyrhynchos* . Can. J. Zool., 49:11-14.

Warner, N. L. and A. Szenberg. 1964. The Immunological Function of the Bursa of Fabricius in the Chicken. Ann. Rev. Microbiol., 18:253-268.

Weydemeyer, W. 1973. The Spring Migration Pattern at Fortine, Montana. Condor, 75:400-413.

Wiens, J. A. 1973. Pattern and Process in Grassland Bird Communities. Ecol. Monogr., 43:237-270.

Wiens, J. A. 1974. Climatic Instability and the "Ecological Saturation" of Bird Communities in North American Grasslands. Condor, 76:385-400.

Wiens, J. A. and G. S. Innis. 1974. Estimation of Energy Flow in Bird Communities: a Population Bioenergetics Model. Ecology. 55:730-746.

Wilson, A. C. and D. S. Farner. 1960. The Annual Cycle of Thyroid Activity in White-Crowned Sparrows of Eastern Washington. Condor, 62:414-425.

Zaccheo, D., C. E. Grossi and V. Genta. 1968. Immunohistochemical Evaluation of the Effects of Surgical and Chemical Bursectomy in the Chicken. III. Intern. Congress of Histochem. Cytochem., New York. pp. 302-303.

Zimmerman, J. L. 1965. Carcass Analysis of Wild and Thermal-Stressed Dickcissels. Wilson Bull., 77:55-70.

Ziswiler, V. and D. S. Farner. 1972. Digestion and the Digestive System. In: D. S. Farner and J. R. King, eds. Avian Biology, Vol. 2. Academic Press, New York. pp. 343-430.

SECTION 23

PARTICULATES IN THE LUNGS OF WESTERN MEADOWLARKS (*STURNELLA NEGLECTA*) IN SOUTHEASTERN MONTANA

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ABSTRACT

The lungs of western meadowlarks (*Sturnella neglecta*), collected within 100 km of the coal-fired power plant and coal mines at Colstrip, Montana, were examined histologically for particulates and associated pulmonary damage. Birds were collected during 1975 (when the power plant was not operating), 1976 (when it operated intermittently), and 1977-78 (when it was in full operation).

Three major categories of particulates occurred in the meadowlark's lung: (1) crystals of variable size, (2) very small (≤0.5 μm) round black flecks, and (3) larger black particles of variable size and shape. Most larger particles were confined to the air ducts, but smaller ones were widespread in the lungs. Crystals were the only particulates that irritated pulmonary tissue, sometimes eliciting mild fibrosis within the parabronchial wall.

Particulates, especially the black forms, increased in the lining of the parabronchi and the lumen of the air capillaries between 1975 and 1977. The number of particulate-containing macrophages in the lining of the parabronchi also increased between 1975 and 1976, but then declined. However, in 1978, the particulate content of the lung was at 1975 levels and there were fewer particulate-containing macrophages in the parabronchi than in 1975. These declines are probably related to the fact that most of the meadowlarks collected in 1978 were obtained at greater distances from Colstrip than birds collected in 1976-77.

Juvenile birds had smaller particulate burdens than adults. Among juveniles collected in 1977 and

1978, the concentrations of particulates were similar in the parabronchi, but significantly different in the air capillaries (smaller in 1978). Particulate burdens of male and female birds were similar in all but one region of the lung. The number of particulate-containing macrophages was negatively correlated with the distance from Colstrip at which a bird was collected during 1975-77.

INTRODUCTION

Birds have the potential to serve as biological monitors of particulate air pollution from coal-fired power plants. Small birds have high metabolic rates and are active at levels where exposure to gaseous and other respirable pollutants may be severe (Lewis and Lewis, 1979). Their lungs are a major site of detoxification and contain macrophages that remove particulate matter from inhaled air. Their pulmonary tissue is easily irritated by inhaled substances to which it exhibits inflammatory responses of greater or lesser severity. In this respect, birds appear to be an order of magnitude more sensitive to aerial pollutants than are humans (Takemoto *et al.*, 1974). Since little respired material is filtered out in the nasal passageways (Takemoto *et al.*, 1974) and air flow through the lung is only in one direction (Bretz and Schmidt-Nielsen, 1971), the avian lung is analogous to a high volume sampler of air-borne materials.

The few published reports concerning the effects of air pollutants on wild birds generally pertain to sedentary urban species and support their use as biomonitors (Lewis and Lewis, 1979). The most striking case of historic importance is the use of the canary (*Serinus canaria*) in sensing mine gases and anoxia (Neal and Olstrum, 1971). Levels of lead in the organs of pigeons (*Columbia livia*), both in Japan and the United States, have been related to aerosol levels of this element at sampling sites (Tansy and Roth, 1970; Ohi *et al.*, 1974). Similar relationships have been discovered between the dust content and associated damage in the lungs of doves and pollutant levels at sampling sites in Japan (Takemoto *et al.*, 1974); and between the presence of particulate-laden pulmonary macrophages in the lungs of house sparrows (*Passer domesticus*) and pollution levels in areas of California (McArn *et al.*, 1974). In addition, Tashiro *et al.* (1974) have demonstrated that birds are especially sensitive to air-borne pollutants during their breeding season. Takemoto *et al.* (1974) found a direct relationship between the age of doves in polluted regions of Japan and the degree of lung damage.

Western meadowlarks (*Sturnella neglecta*) are the most widely distributed and abundant passerine in the Colstrip area (Lewis *et al.*, 1976; Preston and Thompson, 1979). They are neither sedentary nor urban, but presumably return to nest in the same general vicinity year after year (Lewis, unpublished banding returns). Consequently, individuals may be exposed to aerial emissions from the Colstrip power plant for five months each year at a time when they are reproductively active and hence potentially highly sensitive to such insults on the respiratory system. Furthermore, unless they can completely clear particulates from their lungs while absent from Montana (which does not seem to be the case), there may be accumulation of particulates in the respiratory system with successive years of exposure. Under these conditions, western meadowlarks may be particularly useful biomonitors of stack emissions in remote grassland areas such as Colstrip.

MATERIALS AND METHODS

This study deals with samples of lungs from western meadowlarks (*S. neglecta*) collected within 100 km of Colstrip, Montana, between 1975 and 1978. Most birds were taken SSE and SEE of Colstrip, *i.e.*, from sites that are predominantly downwind of the power source (Miller *et al.*, 1976; Crecelius *et al.*, 1978). Birds were shot and dissected in the field as described by Lewis *et al.* (1976). The caudal tip of the left lung was removed from each bird and fixed in Bouin's solution or 10 percent neutral buffered formalin. Fixed samples were later dehydrated, embedded in paraffin, and sectioned at 7.0 µm. Representative sections were stained with haematoxylin and eosin by American Histolabs (Silver Spring, Md.) and examined by us.

Our histological survey of the lung is modeled after methods of McArn *et al.* (1974). Initially, we scanned the sections in order to determine the types of particulates that were present. Then, we examined 20 *transverse* sections of parabronchi and five regions of air capillaries selected at random within each section more closely. We determined the particulate concentrations in (1) macrophages and smooth muscle cells within the lining of each parabronchus, (2) the lumen of each parabronchus, and (3) the lining and lumen of the air capillaries, in each case using a scale of 0 (none present) to 5 (very high concentrations present). In addition, we counted the number of particulate-containing macrophages in the lining of each of the 20 parabronchi.

RESULTS AND DISCUSSION

Histological Structure of the Meadowlark's Lung

We begin with a brief statement concerning the microscopic anatomy of the meadowlark's lung because it has not been described previously. The meadowlark's lung is structurally similar to that of the domestic fowl (Hodges, 1974). However, pulmonary lobules, consisting of a parabronchus and the air capillaries that arise from it, are not as clearly defined as in chickens. There is no conspicuous connective tissue partition between adjacent lobules. In the terminology of Hodges (1974), atria, infundibulae, and air capillaries are all represented in the lobule.

Nature of the Particulates in the Meadowlark's Lung

The lungs of our specimens contained three basic types of particulates (Figure 23.1):

1. Transparent, tetragonal crystals, *ca.* 1.5 µm in length and 0.5 µm in width, scattered individually or in clusters and chains.

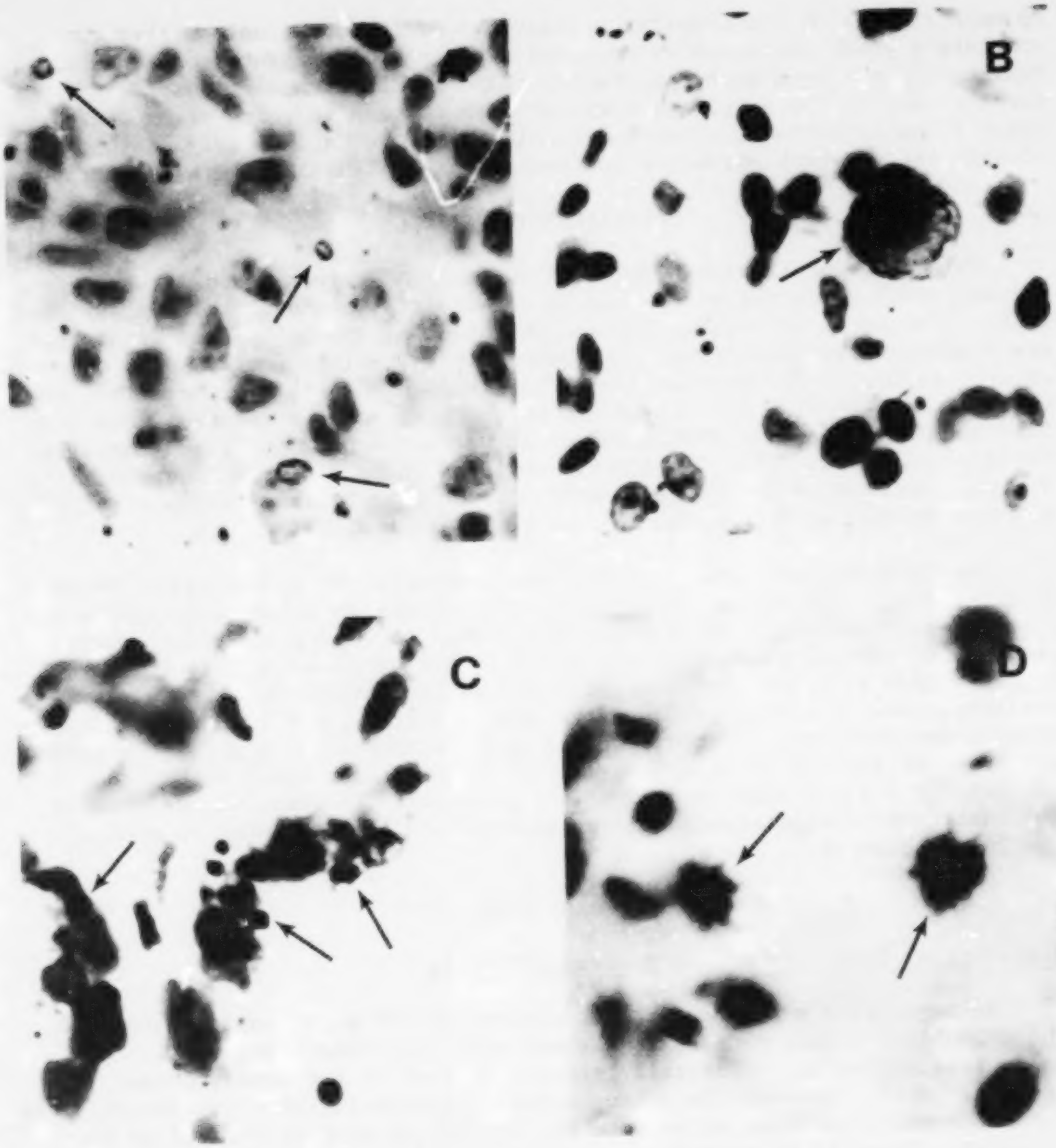

Figure 23.1. Particulates (*arrows*) in the lungs of western meadowlarks collected near Colstrip, Montana.

A and B. Crystalline particulates of small and large size. C. Spherical black particles within macrophages on the border of a parabronchus. D. Large black particulates with distinct projections in an area of air capillaries. Magnification: A - C, 3750X; D, 7500X.

2. Small round black flecks with a diameter <0.5 µm.

3. Larger black particles; these were round, oval, triangular, polygonal, splinterlike, or irregular in shape and varied in size with minimal dimensions between 0.9 and 12.0 µm and maximal dimensions between 1.4 and 21.6 µm. They were sometimes conglomerates of the small flecks.

Black filamentous material and large irregular crystals were also occasionally present. The latter commonly measured between 2.8 and 32.9 µm on a side. Most of the larger particulates were confined to the air ducts, whereas smaller particulates were widespread in both the air ducts and air capillaries.

The parabronchial wall was occasionally thickened around small groups of crystalline particulates. This suggests, as does their size, that they are silicates since (1) the latter are known to elicit fibrotic responses from pulmonary tissue (Bowden, 1976), and (2) one of the major particulates in aerosols near Colstip is an aluminum silicate of a size (0.6 to 1.0 µm) similar to the crystals in the meadowlark lung (Van Valin *et al.*, 1979).

On the basis of their spherical shape and tiny size, the small flecks in the meadowlark's lung may be sulfur- or chlorine-containing particulates emitted by the power plant (Van Valin *et al.*, 1979). These particles and the larger black ones rarely elicited inflammatory responses from the birds' pulmonary tissue aside from occasional small infiltrations of lymphocytes around major blood vessels. Extravasation frequently occurred around heavy concentrations of black particulates, but may be an artifact of the dissection procedure because (1) other heavy concentrations of particulates in the same sections were not enveloped by erythrocytes, and (2) parabronchi were commonly filled with erythrocytes.

Particulate Burdens of the Meadowlark's Lung

The distribution and density of particulates in the meadowlark's lung appear in Table 23.1. To our knowledge, this is the first time that changes in particulate burdens have been examined for several months of each of several years, and with regard to age and sex, in an avian species.

Particulate burdens in the meadowlark's lung increased progressively between 1975 (when the power plant was not operating), 1976 (when it operated intermittently), and 1977 (when it was in full operation). This trend is especially well shown by the particulate content of macrophages in the lining of the parabronchi. However, it is also reflected by changes in particulate density at other sites in the parabronchus, and in the lumen of the air capillaries, although not in the lining of the latter.

All types of particulates increased, but the change in the black forms was especially noticeable (Figure 23.2). Lungs of many adult birds collected in 1975 were completely free of particulates. This was not the case in subsequent years. The change in particulate burdens was especially

TABLE 23.1. PARTICULATE CONTENT OF LUNG TISSUE FROM WESTERN MEADOWLARKS (*Sturnella neglecta*) COLLECTED AT COLSTRIP, MONTANA, BETWEEN 1975 AND 1978*†

		Particulates associated with parabronchi				Particulates associated with air capillaries	
			Particulate density in				
Groups	N	Number of particulate-containing macrophages per parabronchus	Macrophages (rated 0-5)	Smooth muscle cells (rated 0-5)	Lumen of parabronchus (rated 0-5)	Lining of air capillaries (rated 0-5)	Lumen of air capillaries (rated 0-5)
Adults:							
1975	29	24.81 ± 3.99 a	1.38 ± 0.21 a	1.15 ± 0.18 a	0.88 ± 0.16 a	1.93 ± 0.27 a	0.40 ± 0.11 a
1976	41	30.43 ± 4.26 b	2.19 ± 0.24 b	1.31 ± 0.13 a	0.73 ± 0.11 a	1.90 ± 0.18 a	0.40 ± 0.10 a
1977	130-131	22.64 ± 1.06 a	2.45 ± 0.18 b	1.60 ± 0.12 b	1.16 ± 0.09 b	1.95 ± 0.09 a	0.63 ± 0.07 b
1978	27	18.29 ± 1.31 c	1.67 ± 0.26 a	1.08 ± 0.16 a	0.90 ± 0.12 a	1.64 ± 0.11 a	0.47 ± 0.11 ab
Juveniles:							
1977	15	31.05 ± 5.40 a	1.91 ± 0.38 a	1.34 ± 0.29 a	0.98 ± 0.23 a	1.73 ± 0.32 a	0.44 ± 0.09 a
1978	15	27.43 ± 2.74 a	1.46 ± 0.43 a	0.98 ± 0.35 a	0.89 ± 0.26 a	0.88 ± 0.36 b	0.24 ± 0.09 b
Adults:							
April 1976-1977	43	27.20 ± 3.76 a	2.07 ± 0.27 a	1.26 ± 0.19 a	0.88 ± 0.14 ab	1.90 ± 0.21 a	0.60 ± 0.12 ab
May 1975-1977	65	24.27 ± 2.21 ab	2.11 ± 0.23 a	1.39 ± 0.14 a	1.09 ± 0.15(49) bc [1975,1977] 0.62 ± 0.11(16)a [1976]	1.88 ± 0.13 a	0.47 ± 0.10 a

(continued)

TABLE 23.1. (Continued)

Groups		N	Particulates associated with parabronchi				Particulates associated with air capillaries	
			Number of particulate-containing macrophages per parabronchus	Particulate density in				
				Macrophages (rated 0-5)	Smooth muscle cells (rated 0-5)	Lumen of parabronchus (rated 0-5)	Lining of air capillaries (rated 0-5)	Lumen of air capillaries (rated 0-5)
June	1975-1977	68	21.87 ± 1.51(63) b [1975,1977] 33.48 ± 18.00(5)a [1976]	1.28 ± 0.32(9)a [1975] 2.51 ± 0.22(59) b [1976,1977]	1.15 ± 0.16(14)a [1975,1976] 1.88 ± 0.14(54) b [1977]	1.22 ± 0.12 c	2.33 ± 0.37(9)a [1975] 1.76 ± 0.69(5)a [1976] 1.96 ± 0.10(54)a [1977]	0.36 ± 0.18(14)a [1975,1976] 0.65 ± 0.09(54) b [1977]
July	1975, 1977-1978	28	22.64 ± 2.49 ab	1.89 ± 0.45 a	0.85 ± 0.24(13)a [1975,1978] 1.79 ± 0.47(15)ab [1977]	0.97 ± 0.20 abc	1.93 ± 0.27 a	0.51 ± 0.16 ab
August	1975,1978	22	20.77 ± 3.61 b	1.57 ± 0.30 a	1.07 ± 0.20 a	0.87 ± 0.13 abc	1.68 ± 0.14 a	0.46 ± 0.12 ab
Males		171	24.72 ± 1.36 a	2.19 ± 0.18 a	1.43 ± 0.10 a	1.06 ± 0.08 a	1.86 ± 0.09 a	0.43 ± 0.08 a
Females		89	24.53 ± 2.19 a	1.94 ± 0.18 a	1.31 ± 0.12 a	0.90 ± 0.10 b	1.76 ± 0.13 a	0.45 ± 0.07 a
Adults		227	23.61 ± 1.19 a	2.17 ± 0.13 a	1.43 ± 0.08 a	1.02 ± 0.07 a	1.90 ± 0.07 a	0.54 ± 0.05 a
Juveniles		33	31.85 ± 3.20 a	1.67 ± 0.27 b	1.13 ± 0.21 b	0.93 ± 0.15 a	1.30 ± 0.26 b	0.34 ± 0.07 b

*Values in the table are means ± $CI_{95}(N)$. For the monthly changes in the particulate content of the *adult* lung, statistically significant differences existed between years of the study; data were lumped whenever possible, but in some cases were necessarily presented separately.

†Within the column in each division of the table, values *not* followed by the same letter differ significantly at the 0.05 level (Student t-tests and Student-Neuman-Keuls tests for data concerning the number of macrophages; Mann-Whitney or Student-Neuman-Keuls tests for rated data).

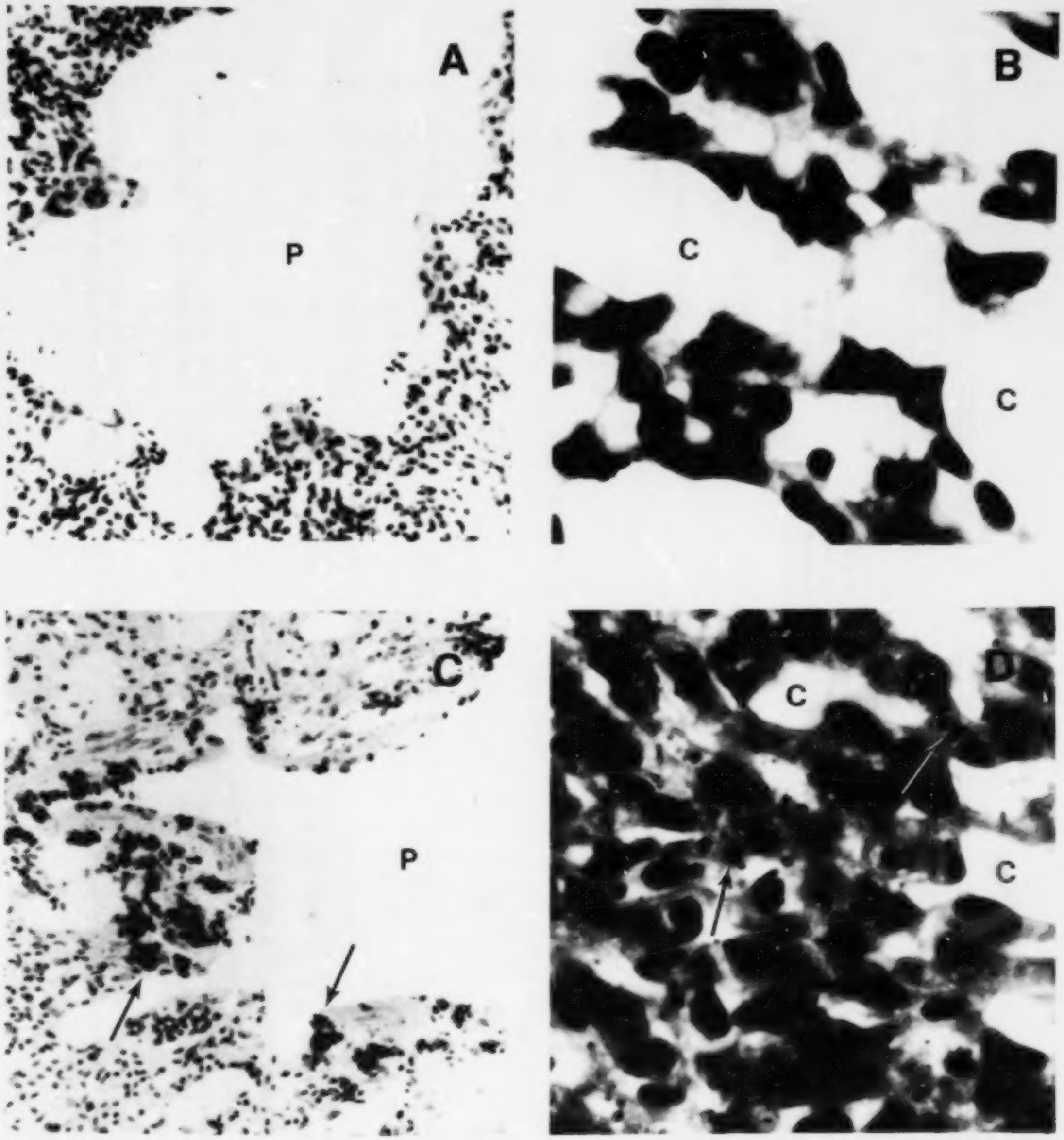

Figure 23.2. Changes in the particulate content of lungs from western meadowlarks collected between 1975 and 1977.

A and B. During 1975, the lining of the parabronchus (*P*) and air capillaries (*C*) was frequently free of particulates. C and D. During 1977, particulates occurred in the lining of both parabronchi and air capillaries (*arrows*). Magnifications: A and C, 750X; B and D, 3750X.

pronounced in macrophages within the lining of the parabronchi. Curiously, the *number* of these macrophages declined in 1977, although the actual particulate content of the parabronchi increased.

In 1978, the particulate content of the lung was similar to that seen in 1975. Furthermore, significantly fewer particulate-containing macrophages occurred in the parabronchi than in 1975. There are several possible explanations for these declines. Most important in this regard is the fact that birds shot in 1978 were intentionally collected at distances in excess of 69 km from the power plant in order to supplement the pre-operational data base for the Colstrip Project. Collections in earlier years were frequently made much closer to the power plant. However, since birds with dirty lungs were collected at these great distances in earlier years, other factors may also be responsible for the decreases in 1978. Perhaps meadowlarks are now actively avoiding heavily impacted areas near the power plant, as suggested by the data of Preston and Thompson (1979), and therefore are exposed to fewer air-borne aerosols than in earlier years. However, we cannot discount the possibility that mechanisms for removing particulates from the lungs have improved (*i.e.*, adapted to the increased particulate burdens) during the 4 years of study. Bowden (1976) has shown, for example, that the number of alveolar macrophages in the mammalian lung is directly related to the number of small particles that reach the alveoli (also see Stuart, 1976). It is also possible that the age structure of the adult segment of the population in 1978 was weighted toward young adults. This may be significant in light of the finding of Takemoto *et al.* (1974) that the trauma produced by aerosols in avian lungs is directly related to the age of birds. We suspect, however, that the declines in 1978 are due largely to the collection procedures followed that year.

Significant differences in the particulate burdens of *adult* lungs existed between the months when the birds were collected. However, we are unable to discern general trends in the data because of differences in some months (*e.g.* June) over the 4 years of study and because monthly changes in one region of the lung are not in the same direction as changes elsewhere in the same lung (Table 23.1). If a small sample of five birds collected in June, 1976, is ignored, it is possible to conclude that the number of particulate-containing macrophages in the parabronchi was higher during April than between May and August.

Significant differences in particulate burdens as a function of age were observed; juvenile birds had cleaner lungs (Table 23.1). Among the juveniles collected during 1977 and 1978, the concentrations of particulates in the parabronchi were similar, but concentrations in the air capillaries differed significantly (lower in 1978). The particulate burdens of male and female meadowlarks were similar in all but one region of the lung that were examined.

Pollution Gradient Analysis

Our study appears to be the first to provide a pollution gradient analysis based on the avian respiratory system. The *number* of particulate-containing macrophages per parabronchus declined as the distance between the site of collection and the power plant increased during 1975, 1976, and 1977, but not during 1978 (Table 23.2). Given the great minimal distance from Colstrip (and the smaller dispersion about the mean) at which meadowlarks were shot during 1978 (69 km), the absence of a relationship during that year is probably not surprising. The inverse relationship between the two variables in each of the earlier years is both linear and significant, although weak (Table 23.2).

Whereas significant relationships existed between the *number* of particulate-containing macrophages and collection distance during 1975-77, only one significant relationship occurred between *particulate density* in the parabronchus or air capillaries and collection distance (Table 23.3). These findings, and the large amount of particulate matter accumulated by pulmonary macrophages (Figure 23.2), suggest that the number of particulate-laden macrophages is the most sensitive indicator of pollution impact on the birds. Data concerning the number of such cells are relatively easy to obtain and may be a sensitive bioindicator of the power plant's impact on birds until and unless the air ducts become saturated with particulates because of repeated exposure to aerosols. Clearly, further information on power plant emissions and air quality in the vicinity of Colstrip is needed and birds may prove useful in its acquisition.

In this regard, it is worth adding that our data are consonant with data obtained with air quality monitoring equipment at Colstrip (Crecelius *et al*., 1978). Using the latter, it has been demonstrated that 69 percent (by weight) of the stack dust at Colstrip is less than 0.3 μm in diameter and hence can remain air-borne for days or weeks and be widely dispersed. A continuously recording air quality monitoring station situated 12 km downwind (SE) from Colstrip has also recorded plume strikes several times each week when the power plant was in operation.

It is possible, but remains to be demonstrated, that the avian lung is a more sensitive and reliable sampler of *low* levels of air-borne particulates than state-of-the-art monitoring equipment. In contrast to the data from meadowlark lungs (Table 23.1), the equipment did not record significant changes in the concentration of elements in Colstrip air after Unit 1 of the power plant began operating.

Recommendations

Since the particulates in the lungs of western meadowlarks at Colstrip may originate from several sources (fugitive dust from traffic on secondary roads or that associated with farming and mining operations; stack emissions of the power plant), it is important to distinguish the impact of the power plant from other potential impacts. Consequently, we recommend that samples of lung be set aside in the future (1) for trace metal analysis, and (2) for examination with the electron microscope, as well as for histological examination.

Atomic absorption analyses of trace metals should indicate if emissions from the power plant are accumulating in the birds' lungs. The stack dust at Colstrip is relatively rich in Ca, Se and V. Concentrations of these elements

TABLE 23.2. DENSITY GRADIENT ANALYSIS: NUMBER OF PARTICULATE-CONTAINING MACROPHAGES PER PARABRONCHUS VS. DISTANCE OF MEADOWLARK FROM COLSTRIP AT CAPTURE

Year	N	Mean distance from Colstrip at capture [km(range)]	Linear relationship between number of macrophages (Y) and distance from Colstrip in km (X)*	Correlation coefficient for the equation (r)	Significance of correlation coefficient
1975	22	74.7 (38.94 - 90.12)	$Y = 57.30 - 0.40\,X$	-0.48	$0.02 < P < 0.05$
1976	44	60.3 (20.92 - 96.56)	$Y = 48.16 - 0.27\,X$	-0.39	$0.005 < P < 0.01$
1977	146	62.4 (18.10 - 96.96)	$Y = 27.92 - 0.07\,X$	-0.25	$0.002 < P < 0.005$
1978	42	74.3 (68.80 - 89.31)	$Y = 17.12 + 0.06\,X$	+0.07	$P > 0.50$

*The slopes (regression coefficients) of these four equations are not statistically different ($P > 0.05$; analysis of covariance)

TABLE 23.3. THE RELATIONSHIP BETWEEN PARTICULATES IN THE LINING OF THE PARABRONCHI AND AIR CAPILLARIES VS. DISTANCE OF THE MEADOWLARK FROM COLSTRIP AT CAPTURE, AS SHOWN BY THE CORRELATION COEFFICIENTS, IS NOT USUALLY LINEAR

		Correlation coefficients (r)	
Year	N	Lining of parabronchus	Lining of air capillaries
1975	22	-0.11	+0.15
1976	44	-0.23	+0.08
1977	146	+0.21*	-0.04
1978	42	+0.18	+0.07

*$0.01 \leq P < 0.02$

(and several others) have been measured in dust at three air monitoring sites near Colstrip (Crecelius *et al.*, 1978). Assays of these elements in samples of lung may more closely establish the contribution of the power plant to the observed particulate burdens in the tissue.

However, atomic absorption analyses will not indicate the distribution of these and other particulates in the lungs nor the damage they cause to pulmonary tissue. Hence, routine histological surveys should be continued. They should be complemented with ultrastructural studies, similar to those done by McArn *et al.* (1974), in order to clearly identify particulates of very small size in the lung.

One limitation of the study to-date involves the method used to fix the lungs. Extravasation is commonly widespread in the organ and fixation is not uniformly good. These shortcomings confound histological evaluation and can be readily avoided if in the future the air duct system of the bird is perfused with fixative before the lung is removed and fixed. This method of fixation will also preserve the larger ducts in the respiratory tree. It would be useful to study the effects of particulates on the mucociliary apparatus of these larger airways since they are known to be affected by exposure to such things as SO_2 in other species of birds (Wakabayashi *et al.*, 1977). In addition, a considerable fund of information concerning the structure of these larger airways of birds, both at the light and electron microscopic levels, is already available in the literature and therefore readily available for comparison (Bienenstock *et al.*, 1973; Hodges, 1974; Walsh and McLelland, 1974 a,b; Jeffery, 1978).

CONCLUSIONS

The meadowlark's lung is structurally similar to that of the domestic fowl.

Three major categories of particulates occurred in the meadowlark's lung during 1975-78: (a) crystals of variable size, (b) small ($\leqslant$ 0.5 μm) round black flecks, and (c) larger black particles of variable size and shape. The crystals may be silicates. The small flecks may be sulfur- or chlorine-containing particles emitted by the power plant. Larger particles were generally confined to the air ducts, but smaller ones were widespread in the lungs. Only the crystals caused perceptible irritation to the lung.

All categories of particulates, but especially the black forms, increased in the lining of the parabronchi and the lumen of the air capillaries between 1975 and 1977. The number of particulate-containing macrophages in the lining of the parabronchi also increased between 1975 and 1976, but then declined. In 1978, the particulate content of the lung was at 1975 levels and there were fewer particulate-containing macrophages in the parabronchi than in 1975. These declines are probably related to the fact that most of the meadowlarks collected in 1978 were obtained at greater distances from Colstrip than birds collected in 1976-77.

Significant monthly variations in particulate burdens occurred at the various sites of the lung that were examined, but overall monthly trends are difficult to discern. However, the number of particulate-containing macrophages was, with one exception, higher during April than between May and August.

Juvenile birds had smaller particulate burdens than adults.

Among the juveniles collected in 1977 and 1978, the concentrations of particulates were similar in the parabronchi, but significantly different in the air capillaries (smaller in 1978).

Particulate burdens of male and female birds were similar in all but one region of the lung that were examined.

The *number* of particulate-containing macrophages was significantly and negatively correlated with the distance from Colstrip at which a bird was collected during 1975, 1976, and 1977.

Only one significant relationship (weak and positive) occurred between *particulate density* in the lung and the distance from Colstrip at which a meadowlark was collected.

Of the histological measurements, the number of particulate-laden macrophages in the lining of the parabronchi appears to be the most sensitive indicator of pollution impact on meadowlarks. It appears to be a useful biomonitor of the particulate output of the coal-fired power plant at Colstrip.

REFERENCES

Bienenstock, J., N. Johnston, and D.Y.E. Perey. 1973. Bronchial Lymphoid Tissue. I. Morphologic Characteristics. Lab. Invest., 28(6):686-692.

Bowden, D.H. 1976. The Pulmonary Macrophage. Env. Health Persp., 16:55-60.

Bretz, W.L., and K. Schmidt-Nielsen. 1971. Bird Respiration: Flow Patterns in the Duck Lung. J. Exp. Biol., 54:103-118.

Crecelius, E.A., L.A. Rancitelli, and S. Garcia. 1978. Power Plant Emissions and Air Quality. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains, DOE Annual Report, September, 1978, (unpublished), J.J. O'Toole and C.C. Gordon, eds. pp. 9-33.

Hodges, R.D. 1974. The Histology of the Fowl. Academic Press, Inc., New York. 648 pp.

Jeffery, P.K. 1978. Structure and Function of Mucous-Secreting Cells of Cat and Goose Airway Epithelium. Ciba Foundation Symp., 57:5-23.

Lewis, R.A., and C.W. Lewis. 1979. Terrestrial Vertebrate Animals as Biological Monitors of Pollution. In: Monitoring Environmental Materials and Specimen Banking, N.-P. Luepke, ed. Martinus Nijhoff Publ., The Hague. pp. 369-391.

Lewis, R.A., M.L. Morton, and S.C. Jones. 1976. The Effects of Coal-Fired Power Plant Emissions on Vertebrate Animals in Southeastern Montana (A Report of Progress). In: The Bioenvironmental Impact of A Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana, June, 1975, R.A. Lewis, N.R. Glass, and A.S. Lefohn, eds. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 140-187.

McArn, G.E., M.L. Boardman, R. Munn, and S.R. Wellings. 1974. Relationship of Pulmonary Particulates in English Sparrows to Gross Air Pollution. J. Wildlife Dis., 10:335-340.

Miller, J.R., T.Cail, and A.S. Lefohn. 1976. Air Monitoring Characterization at the Hay Coulee Site, Colstrip, Montana. In: The Bioenvironmental Impact of A Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana . R. A. Lewis, N. R. Glass, and A.S. Lefohn, eds. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 213-221.

Neal, J.E., and E.G. Olstrum. 1971. Birdlife - An Indicator of Environmental Quality. Ext. Bull. E-707, Natural Resources Series, Cooperative Extension Service, Michigan State Univ., East Lansing, Mich. 4 pp.

Ohi, G., H. Seki, K. Akiyama, and H. Yagyu. 1974. The Pigeon, A Sensor of Lead Pollution. Bull. Env. Contamination Tox., 12(1):92-98.

Preston, E.M., and S.K. Thompson. 1979. Trends in Bird Populations in the Vicinity of Colstrip. In: The Bioenvironmental Impact of A Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E.M. Preston and T.L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 240-256.

Stuart, B.O. 1976. Deposition and Clearance of Inhaled Particles. Env. Health Persp., 16:41-53.

Takemoto, K., H. Katayama, K. Namie, R. Endo, and K. Tashiro. 1974. Effects of Air Pollution on the Ornitho-Respiratory System. Part IV. Pathology of Doves Lung. Jap. J. Hyg., 29(1):106.

Tansy, M.F., and R.P. Roth. 1970. Pigeons: A New Role in Air Pollution. J. Air Poll. Cont. Ass., 20(5):307-309.

Tashiro, K., K. Namie, K. Takemoto, and E. Hisazumi. 1974. Effects of Air Pollution on the Respiratory System of Animals in a Zoological Garden. Jap. J. Hyg., 29(1):107.

Van Valin, C.C., R.F. Pueschel, D.L. Wellman, N.L. Abshire, G.M. Lerfald, and G.T. McNice. 1979. Aerosol Characterization in the Vicinity of Colstrip, Montana. In: The Bioenvironmental Impact of A Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E.M. Preston and T.L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 2-52.

Wakabayashi, M., B.G. Bang, and F.B. Bang. 1977. Mucociliary Transport in Chickens Infected with Newcastle Disease Virus and Exposed to Sulfur Dioxide. Arch. Env. Health, 32(3):101-108.

Walsh, C., and J. McLelland. 1974a. The Ultrastructure of the Avian Extrapulmonary Respiratory Epithelium. Acta Anat., 89:412-422.

Walsh, C., and J. McLelland. 1974b. Granular "Endocrine" Cells in Avian Respiratory Epithelia. Cell Tissue Res., 153(2):269-276.